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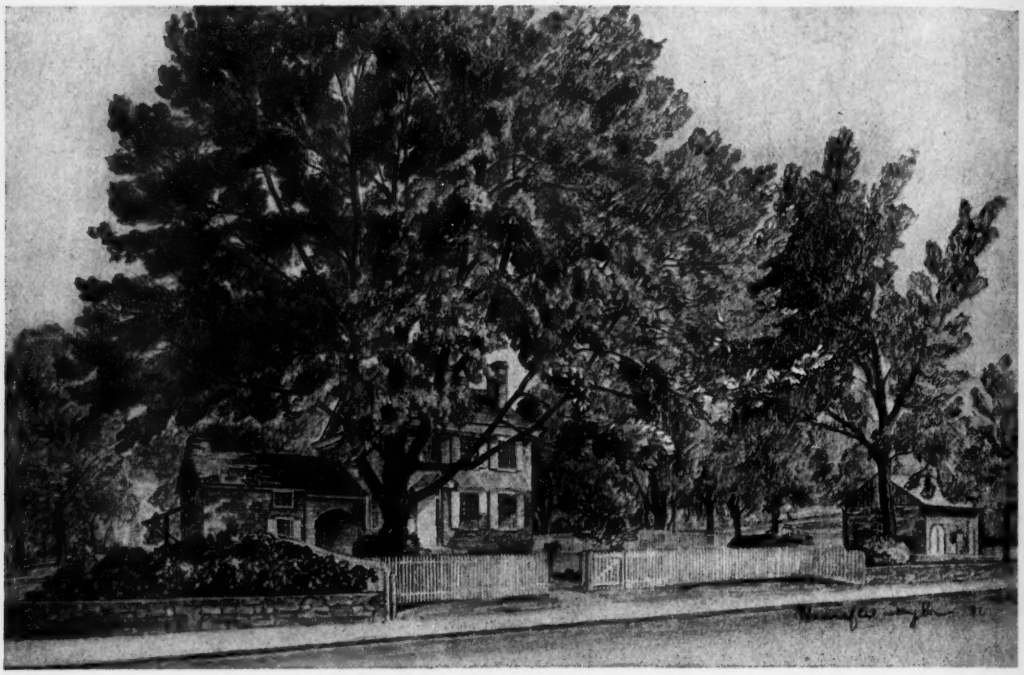

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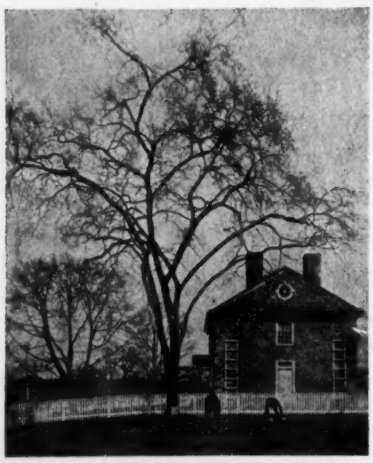

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An International Journal
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PHYTOPATHOLOGY

VOLUME 21 NUMBER 11

NOVEMBER, 1931

AN EXPERIMENTAL INVESTIGATION OF SEX IN THE RUST FUNGI[1]

J. H. CRAIGIE

INTRODUCTION

The rusts comprise a distinct group of the Basidiomycetes. They are all parasitic, and some of them may, under favorable conditions, cause destructive epidemics. On account of their obligate parasitism, they present considerable difficulties to cultural studies. Owing to this fact, possibly, certain aspects of their developmental cycle have remained obscure. They have, however, been the subjects of a great deal of investigation. The black stem rust of cereals has been studied more than any other rust fungus. In general, it may be said that the amount of study given to a particular species has been about proportional to its economic importance.

This paper is a contribution to our knowledge of the rust fungi, particularly in respect to their sexuality; and the observations about to be recorded have been made on *Puccinia graminis* Pers., *P. helianthi* Schw., *P. coronata* Corda, *P. Pringsheimiana* Kleb., and a species of Gymnosporangium.

HISTORICAL SUMMARY

Our knowledge of the life history of rust fungi made a great advance in the middle of the nineteenth century. Previous to that time each spore form was regarded as belonging to a distinct genus. Tulasne (79), in 1854, showed that the uredinial and telial sori that are found on stems of wheat are not derived from two rust species, as had previously been believed, but that they are products of the mycelium of one and the same species, namely, *Puccinia graminis*. In 1865, de Bary (17) established experimentally the genetic connection of the two stages of the rust fungus on wheat with the aecial stage (*Aecidium berberis* Pers.) on the barberry. He inoculated barberry plants with sporidia from germinating teliospores. From these inoculations arose pycnia and aecia. Wheat plants inoculated with the aeciospores produced uredinia and telia. Thus he demonstrated

[1] Contribution from the Division of Botany, Department of Agriculture, Ottawa, Canada. This memoir was awarded the Eriksson Prize for Cereal Rust Investigations, 1930. It was submitted to the University of Manitoba in the spring of 1930 as a thesis in partial fulfilment of the requirements for the Ph.D. degree.

that *P. graminis* produces five spore forms which appear in regular sequence: pycniospores in pycnia; aeciospores in aecia; urediniospores in uredinia; teliospores in telia; and sporidia on the basidium derived from a germinating teliospore.

Pycnia were discovered by Unger (81) in 1833 and were considered by him to be the fructification of a distinct fungus. Since that time the pycnia have been the object of much speculation. Meyen (50) noticed the close association of pycnia and aecia and suggested that they represented the sexual organs of one and the same fungus. This view was supported by Tulasne (80). As the pycniospores were apparently lacking in power of germination and as the pycnia bear a striking resemblance to structures in collemaceous lichens, believed to be sexual in nature, Tulasne (78) called the pycnia spermogonia. De Bary (16) inclined to the view that the spermogonia of the rust fungi are male organs, producing spermatia which are apparently incapable of germination; but he pointed out that pycnia (spermogonia) are unaccompanied by aecia in some rusts, and, in some other rusts, aecia are unaccompanied by pycnia. He therefore considered it best, until further knowledge became available, to regard the pycnia as organs whose physiological significance was doubtful. The germination of pycniospores of rust fungi was observed by Cornu and Roze (11), and the germination of the spermatia of lichens by Möller (51). They regarded these spore forms as conidia. The fact that pycniospores had been observed to germinate and that aecia are sometimes unaccompanied by pycnia led Brefeld (8) to oppose the idea of sexuality in the rusts and to consider the pycnia not as spermogonia but as asexual reproductive organs to which the name pycnidia should be applied. Klebahn (33) opposed the view that the pycnia are male organs but pointed out that the pycniospores do not behave like conidia. Plowright (61) arrived at much the same conclusion. If the pycniospores are conidia, they should, in his opinion, be able to infect the host plant, but he was never able to prove such infections experimentally. However, he observed the germination of pycniospores in sugar solutions.

The opinion of Brefeld that the pycnia are not male organs was more or less generally accepted until 1904, when Blackman (6) revived the theory of the sexual nature of the pycnia. He supposed that the pycniospores were once functional, like the spermatia of the red seaweeds, but that they became functionless when the trichogynes of the rusts disappeared. In support of his view that pycniospores are functionless male cells, he referred to the density of their nuclei, the paucity of their cytoplasm, and the thinness of their cell wall. In addition, he pointed out that pycniospores possess but feeble power of vegetative development and are (as far as known) incapable of producing infection. Christman (10) in-

clined to the view that the pycnia once produced functional asexual gametophytic spores—conidia—as do the pycnidia of Ascomycetes. He questioned the belief that the pycniospores are functionless, although he admitted that their nature was not very evident. It was McAlpine's (47) belief that the pycnia are isolated organs and that, whatever their original function may have been, they are now quite functionless. Grove (25) advanced eight reasons in support of the assumption that the pycniospores represent functionless male cells. Later investigators accepted one or other of these two theories, but the opinion was fairly unanimous that the pycnia, whether they were originally asexual gametophytic reproductive organs or sexual organs producing spermatia, are no longer functional. The view that the pycniospores are functionless was very definitely expressed by Gwynne-Vaughan and Barnes (26) in their text-book published in 1927.

Cytological investigations have made important contributions to our knowledge of the rust fungi. In 1880, Schmitz (67) discovered paired nuclei in the mycelium and urediniospores of *Coleosporium campanulae* (Pers.) Lév. Two nuclei were seen by Rosen (64) in the aeciospores of *Uromyces pisi* (Pers.) de Bary and in the young teliospores of *Puccinia asarina* Kunze but only one in the mature teliospores. Poirault and Raciborski (62) introduced the term "conjugate nuclei" for the paired nuclei and made some observations on the chromosomal behavior during nuclear fusion and reduction. A comprehensive study of the mycelia and fructifications of a large number of rust species was made by Sappin-Trouffy (66). He found that the pycniospores and the hyphae which produce them are uninucleate; that the aeciospores, urediniospores, and the hyphae arising from them are binucleate, as are also the cells of the immature teliospores, but that, before germination, the cells of the teliospores become uninucleate through the fusion of their conjugate nuclei. Two divisions occur in the basidium, one of which is a reduction division, so that the four basidiospores are uninucleate and produce uninucleate mycelia. He considered that the fusion of the two nuclei in the teliospore represents a true sexual process but paid little attention to the origin of the binucleate condition in the aeciospores.

The origin of the binucleate condition was investigated by Blackman (6), who found that in *Phragmidium violaceum* Wint. the nucleus of one cell migrates through a pore into a neighboring cell. He interpreted this as the beginning of a sexual act which culminates in the fusion of the two nuclei in the teliospore. Christman (10), in a similar study on *P. speciosum* Burrill, found that fusion occurs between two neighboring cells and suggested that the nuclear migration observed by Blackman might have been due to a pathological condition. Nuclear migration also has been observed by Welsford (89), Kursanov (38), and several others; but

most of the later workers, Olive (58), Kursanov (37), Fromme (23), Maire (46), Lindfors (42), etc., have found that the conjugate condition of nuclei in the spore bed of the aecia of many rust species is due to cell fusion, although both types have been found in some species (38, 42, 58). Very recently, Hanna (29) has shown that cell fusion occurs in *Puccinia graminis*.

The phenomenon of heterothallism is known to occur in the Phycomycetes, the Ascomycetes, and the Basidiomycetes. It has been demonstrated experimentally: in the Phycomycetes, by Blakeslee (7) in Mucor and other Mucorineae, and by Couch (12) in Dictyuchus; in the Ascomycetes, by Dodge (20) in *Ascobolus magnificus* Dodge, by Betts (5) in *A. carbonarius* Karst., by Derx (18) in *Penicillium,* by Wieben (90) in *Taphrina,* and by Shear and Dodge (69) in *Neurospora sitophila* Shear & Dodge and *N. crassa* Shear & Dodge. Dodge (21) secured fertile hybrids from a cross between *N. sitophila* and *N. tetrasperma* Shear & Dodge.

In the Hymenomycetes heterothallism has been shown to occur: by Mlle. Bensaude (4) in *Coprinus fimetarius* (Lin.) Fr.; by Kniep (34, 36) in *Schizophyllum commune* Fr. and *Aleurodiscus polygonius* (Pers.) v. Hoehn. & Litsch; by Miss Mounce (53, 54) in *Coprinus lagopus* Fr., *C. niveus* Fr., and *Fomes pinicola* Swartz; by Vandendries (82, 83, 84) in *Collybia velutipes* Court., *Hypholoma fasiculare* Huds., *Panaeolus campanulatus* L., *P. separatus* L., *P. fimicola* Fr., and *Coprinus radians* Desm.; by Brunswik (9) in a number of species of Coprinus; by Miss D. E. Newton (55) in *Coprinus Rostrupianus* Hansen; and by Miss Gilmore (24) in *Psilocybe coprophila* Bull.

Among the smut fungi, Kniep (35) found evidence of heterothallism in *Ustilago violacea* Pers.; Stakman and Christensen (71), in *U. zeae* (Beckm.) Ung.; Dickinson (19) in *U. levis* (Kell. & Sw.) Magn. and *U. hordei* (Pers.) Kell. & Sw.; and Hanna (28) in *U. zeae* and *Sorosporium reilianum* (Kühn) McAlp.

Until 1894, *Puccinia graminis* was considered to be a single species capable of attacking all of the common cereals and grasses, but in that year Eriksson (22) showed that it consisted of several pathogenic strains or forms, each of which is specific for certain gramineous hosts. These strains he designated as "formae speciales" and classified them according to their infective capabilities as follows: *P. graminis tritici* Eriks. & Henn. on wheat, *P. graminis avenae* Eriks & Henn. on oats, *P. graminis secalis* on rye, *P. graminis airae* Eriks. & Henn. on Aira, *P. graminis agrostis* Eriks. & Henn. on Agrostis, and *P. graminis poae* Eriks. & Henn. on Poa. All had the common barberry as an aecial host. Results of a confirmatory nature were obtained by Rostrup (65), Magnus (43), Hitchcock and Carleton (30), Klebahn (32), and Ward (87).

A careful study by Stakman and Piemeisel (76) in 1917 revealed that *Puccinia graminis tritici* was not a simple form but consisted of at least 2 pathogenic strains. In the following year, a third strain was identified by Melchers and Parker (49) and a fourth one by Levine and Stakman (40). By using twelve standard varieties of wheat, called "differential hosts," Stakman and his coworkers (72, 75, 77) have been able to distinguish a large number of pathogenic forms by their reaction on these hosts. Newton, Johnson and Brown (56) have reported 8 additional forms. As the morphological characters of all these strains are more or less identical, the dissimilarity of their reactions is considered to be due to physiological differences in the strains, and, hence, the strains are now referred to as "physiologic forms."

Not only has physiologic specialization been found in *Puccinia graminis tritici,* but it has been demonstrated by Stakman, Levine, and Bailey (73) in *P. graminis avenae* and by Levine and Stakman (41) in *P. graminis secalis.* It has also been shown to occur in other rusts. Mains and Jackson (45) distinguished 12 forms in *P. triticina* Eriks.; Mains (44), 4 forms in *P. sorgi* Schw. and 2 in *P. anomala* Rostr.; Hoerner (31), 4 forms in *P. coronata* Corda; and Bailey (3), at least 3 forms in *P. helianthi* Schw. These citations are sufficient to indicate that the phenomenon of physiological specialization occurs more or less generally among the rust fungi.

From the historical summary just concluded, it is evident that prior to 1926, at which time the investigation here recorded was begun, many fundamental facts concerning the life history of *Puccinia graminis* had been brought to light; it was known that this rust is heteroecious; that fusion of the conjugate nuclei occurs in the teliospores prior to germination; that reduction takes place in the basidium; that the sporidia give rise to uninucleate mycelia; and that the aeciospores are binucleate. By inference from what was known to occur in other rusts, the binucleate condition of the aeciospores was supposed to arise either by cell fusion or nuclear migration in the aecial spore bed. It was known also that physiological specialization in *P. graminis* is very pronounced. And, finally, it was generally agreed that, whatever the original function of the pycnia may have been, that function had been lost.

No one up to the year 1927 ever had studied the problem of sex in *Puccinia graminis* or any other rust experimentally; but, in that year, the writer was able to announce that *P. graminis* is heterothallic and that the pycnia are functional (13, 14). A further communication from the writer (15) in 1928 contained additional observations on sex in the rust fungi.

PROBLEM STATED

Black stem rust (*Puccinia graminis*) is one of the most destructive diseases of cereal crops. As already stated, *P. graminis* is not a simple

species but comprises a large number of physiologic forms. The fact that one cereal variety may be highly resistant to one or more physiologic forms but quite susceptible to other forms has very materially impeded the plant breeder in his effort to produce rust-resistant varieties of cereals. Whether or not new physiologic forms arise from time to time under natural conditions was not known, but it was argued theoretically that if *P. graminis* is heterothallic any two physiologic forms of this organism may hybridize on the barberry and so produce new forms which may render breeding for rust resistance more or less futile. Stakman, Levine, and Leach (75), as early as 1919, mentioned the possibility of hybridization of physiologic forms occurring on the barberry. Whether or not hybridization does take place is a question of very practical importance, as well as one of considerable scientific interest. The writer undertook to investigate this problem.

It seemed obvious to him that the first step in the investigation was to discover whether *Puccinia graminis* is homothallic or heterothallic. Both homothallic and heterothallic species are known among the Basidiomycetes. No sexual differentiation was found by Miss Mounce (52) to exist in the basidiospores of *Coprinus sterquilinus* Fr. or of *C. stercorarius* Gillet, or by Brunswik (9), in the basidiospores of *C. narcoticus* Fr. or of *C. ephemeroides* Fr. On the other hand, Kniep (35) showed that the basidiospores of *Ustilago violacea* are divisible into two sexual groups, and a similar condition was demonstrated by Vandendries (84) in *C. radians* and by Miss D. E. Newton (55) in *C. Rostrupianus*. Four sexual groups of spores were found by Kniep (34, 36) in *Schizophyllum commune* and *Aleurodiscus polygonius*, and by Hanna (27) in *C. lagopus*. It was therefore possible that one of these three conditions might exist in *P. graminis*.

If the sporidia of this rust are not differentiated for sex, or, in other words, if the species is homothallic and its sporidia are all sexually alike, an infection of a barberry leaf by a single sporidium should produce a pustule in which normal aecia would arise. If the sporidia were differentiated for sex, or, in other words, if the species is heterothallic and its spores are divisible sexually into 2 or 4 groups, an infection by a single sporidium should produce a mycelium which would remain in the haploid condition. Aecia would not be expected to develop, therefore, in such a pustule, unless indeed, a spontaneous change from the haploid to the diploid condition occurs, similar to that found by Vandendries (85, 86) in *Coprinus radians* and *C. micaceus* (Bull) Fr., and by Miss D. E. Newton (55) in *C. Rostrupianus*. Aecia might be expected, however, to arise in a compound pustule formed by the coalescence of two monosporidial pustules of opposite sex, but not in a compound pustule formed by two coalescing pustules of the same sex or of two sexes incapable of interacting sexually.

In attempting to elucidate the sexual condition of *Puccinia graminis* from the point of view of the possibilities just discussed, it was necessary to obtain two kinds of pustules: (1) simple pustules, each derived from the sowing of a single sporidium and (2) compound pustules, each derived from the sowing of two sporidia very close to each other and from the eventual coalescence of the two simple pustules resulting therefrom.

MATERIALS

As the primary object of the work was a study of the sexual behavior of *Puccinia graminis*, this rust was used throughout the investigation, and exclusively in 1926. For reasons that will be mentioned later, *P. helianthi* was used extensively in the experiments of 1927. The source of the telial material of *P. graminis* was heavily infected culms of wild barley, *Hordeum jubatum* DC., and that of *P. helianthi*, withered leaves of the cultivated sunflower, *Helianthus annuus* L. Collections of both rusts were made at the Agricultural College, Winnipeg, Manitoba.

For convenience in handling, only small plants of the respective hosts were employed. The barberry plants varied from 6 to 12 in. in height and grew singly in 6-in. flower pots. Sunflower seedlings were grown in similar pots, usually 4 or 5 to a pot. They were inoculated as soon as the first foliage leaves were 1 in. in length.

Owing to the fact that barberry leaves become very highly resistant to infection after they are 12 days old, only young leaves of this plant were successfully inoculated. Moreover, as a leaf grows older, it appears to offer progressively greater resistance to the spread of the mycelium through its tissues. The older the leaf when infected, the smaller usually is the pustule that arises from the infection.

METHODS

Inoculations were made by two methods, A and B. By method A, sporidia were picked off their sterigmata and deposited, either singly or in pairs, in a drop of water on the leaf of the host plant. By method B, sporidia were sown sparsely over the leaves of the host plant.

Method A was employed exclusively during 1926. It was not, however, very productive of results, but as it was the method by which the first results were obtained and as, in the opinion of the writer, the comparative failure attending its employment was not inherent in the mechanism or manipulation of the spore-picking apparatus but attributable to another factor, a description of the apparatus and its operation will be given in detail.

(1) *Method A*

Before inoculations could be made by this method, it was necessary to construct a spore-picking device which was amenable to rather quick

manipulation. A drop of water begins to form at the base of a sporidium just about 15 seconds before the sporidium is discharged. The formation of this drop indicates that the sporidium is mature. Only mature sporidia were desired for inoculating barberry leaves. Contact had to be made with a sporidium while its drop was being excreted, *i.e.*, within a period of 15 seconds. Also, a moist chamber was required in which an optimum humidity for teliospore germination could be maintained and still permit ready access to an instrument to pick off the sporidia.

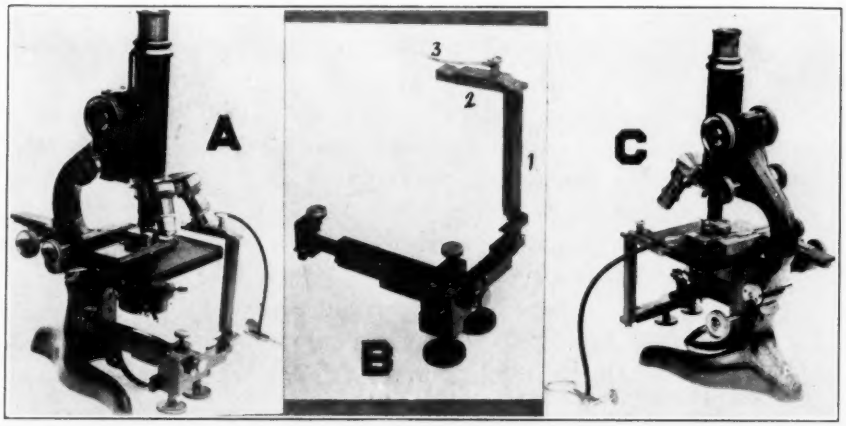

FIG. 1. A. View of right-hand side of microscope showing one mechanical stage in the usual position and another inverted and clamped to the projection on the substage which supported the iris diaphragm. B. View of mechanical stage in inverted position showing: the upright pillar, *1;* the horizontal arm, *2*, with its curved notch (towards the left end); and the spring clip, *3*. The stage is ready to be attached to the microscope. C. View of left-hand side of microscope showing capillary tube with attached rubber tubbing in position and ready for use.

Apparatus. The iris diaphragm was removed from a Leitz microscope and to the projection which supported the diaphragm was clamped in an inverted position a mechanical stage (Fig. 1, A). The swing arm of the stage was disjoined and at its place of attachment was erected a wooden pillar, 1, as shown in fig. 1, B. At the top of 1, a short wooden arm, 2, was fixed at right angles. On the upper side of 2, a spring clip, 3, was firmly secured.

A short piece of glass tubing, 5 mm. in diameter, was drawn out to a fine capillary bore. The unreduced part was fitted into the end of a piece of soft rubber tubing about 8 in. in length and of the same diameter as the glass tube. Thus connected, the two were mounted as seen in figure 1, C. The part of the glass tube covered by the rubber tubing was placed in the notch (Fig. 1, B) cut on the upper side of 2 and was held in position by

the spring clip, 3. The capillary part of the tube reached to the proximal edge of the field of view of the microscope; and the free end of the rubber tubing, closed by a spring clamp, rested on the table (Fig. 1, C).

It was then possible to raise or lower the capillary tube by means of the rack and pinion which served to adjust the substage of the microscope; and to move it from right to left, or from front to rear, within limits, by the inverted mechanical stage.

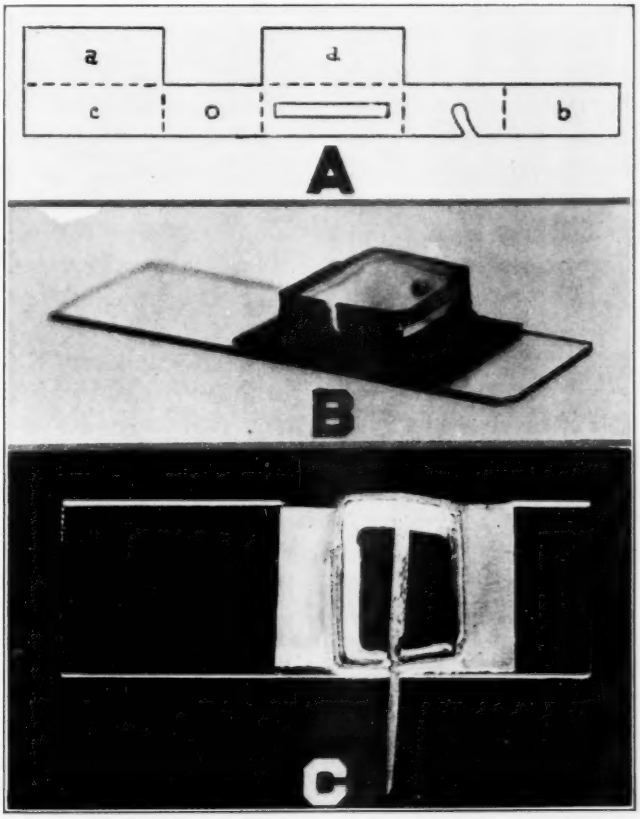

FIG. 2. A. Pattern to which a piece of Bristol board was cut to form the walls of a moist chamber. The Bristol board was folded along the dotted lines so that the two wings, marked *a*, projected outward and lay flat on the surface of the glass slide. The narrow end, marked *b*, overlapped the corresponding portion of the other end, marked *c*, and was glued to it. Slightly reduced. B. Moist chamber showing rectangular opening in front to the right, the notch and hole in the two side walls, and the filter-paper pad. Slightly reduced. C. Top view of moist chamber showing the filter-paper lining (white) and the piece of straw bearing telia mounted on the pin and ready for use. Actual size.

To form a moist chamber, a heavy strip of Bristol board was cut to the pattern shown in figure 2, A, and folded along the dotted lines; so that, when completed and cemented to a glass slide, it presented the appearance shown in figure 2, B. Before being cemented to the slide, it was dipped in liquid paraffin to prevent water absorption when in use. The rectangular opening in the front (Fig. 2, B) afforded access to the interior. A filter-paper pad lined the interior of the other three sides. Midway across the chamber lay a small wooden pin (Fig. 2, C), supported at one end by a small hole in one side wall of the chamber and, at the other end, by the notch cut in the opposite side wall. The pin projected about half an inch beyond the side of the chamber so that it could easily be reached by the hand and be rotated by the thumb and finger.

Preparation of inoculum. A straw bearing numerous telial sori was soaked in water for about 1 hour. From it was cut off a piece just long enough to reach across the moist chamber. The wooden pin was passed through the hollow center of the piece of straw and was then replaced in the chamber, as shown in figure 2, C. Sufficient water was added to the filter-paper pad to wet it thoroughly. Thus both ends of the piece of straw were in contact with water, and, so long as the filter-paper remained wet, the telial sori were kept damp. To prevent the mount from drying during the ensuing night, the moist chamber (all the apparatus shown in figure 2, C) was set in a Petri dish, the bottom of which was covered with water.

To secure basidia which projected sufficiently from the straw, it was necessary to keep the telial sori covered with a thin film of moisture. No exact data on the optimum moisture requirement were taken. Experience alone served as guide. But when a certain thickness of film was present, the basidia were somewhat longer and stood well out from the sori, in which case it was easy to bring the capillary tube into contact with individual sporidia. If too much moisture was present, the basidia were distorted and produced abnormal sterigmata and sporidia.

Manipulation. When the teliospores in the sori began to germinate, the moist chamber was removed from the Petri dish and covered with a cover glass. The chamber, thus completed, was then mounted under the microscope on the regular mechanical stage which had previously been moved somewhat to the right to prevent the chamber, when being mounted, from coming into contact with the end of the capillary tube. The capillary tube was then removed from its position and filled about two-thirds full with distilled water. This was done by releasing the end of the rubber tubing from the spring clamp and then by drawing the thumb and index finger of the right hand along the tubing, thereby creating a partial vacuum in the tubing, while the capillary end of the tube was held by the left hand under water. When the desired amount of water was drawn up, the spring

clamp was replaced and the tube mounted again in its former position, the capillary end of the tube appearing at the edge of the microscopic field (Fig. 3).

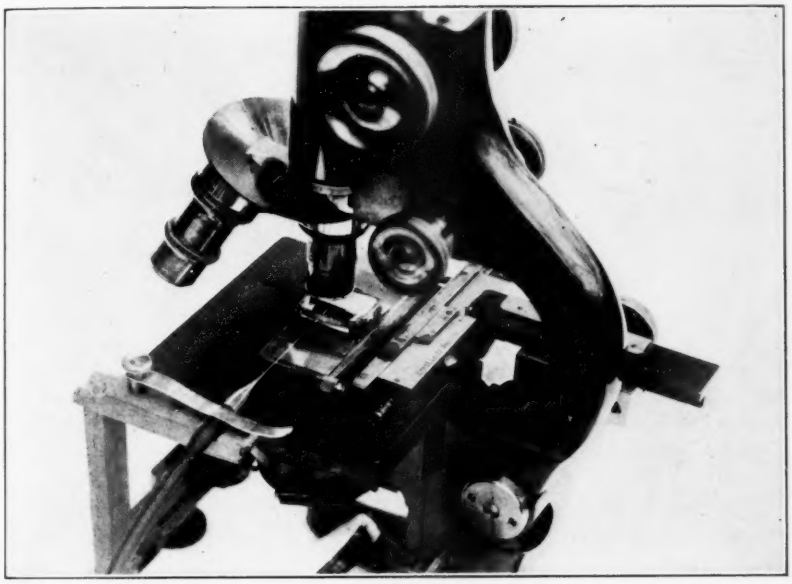

FIG. 3. View of middle portion of microscope showing moist chamber (cover glass removed) and capillary tube in position. The end of the capillary tube has passed through the rectangular opening of the moist chamber and has approached the mount of telium-bearing straw which is supported by the wooden pin.

By means of the mechanical stage, the moist chamber was moved to the left until the piece of straw bearing the telia appeared at the side of the field of view opposite that occupied by the end of the capillary tube, the latter having been adjusted for height so that it passed freely through the rectangular opening in the side of the chamber (Fig. 3). Search was then made for a somewhat isolated basidium bearing a sporidium which was approaching maturity. When one was found, it was brought directly opposite the end of the capillary tube (Fig. 4, A), but not close up to it, so that other sporidia which might be discharged while this one was maturing would not come into contact with the tube. If by any chance an unwanted sporidium did come into contact with the tube, the tube was removed, sterilized in boiling water, refilled with distilled water, and put back into position again.

Whenever the drop of water which forms at the base of the sporidium first became visible, the mount and capillary tube were moved towards one

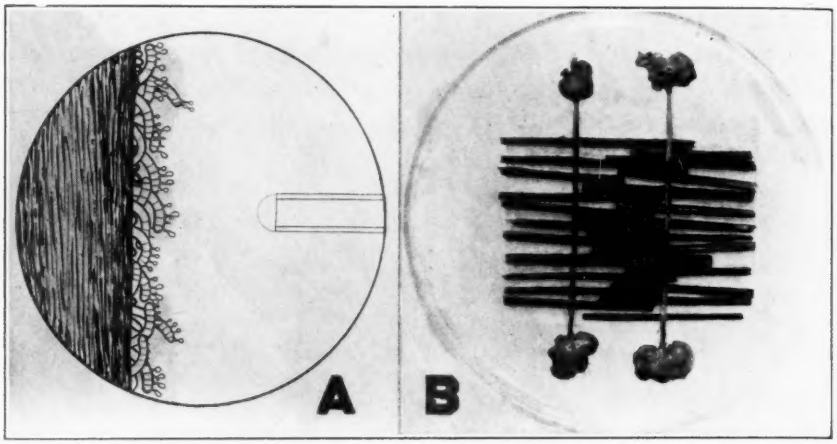

FIG. 4. A. Diagrammatic representation of a microscopic field showing the end of the capillary tube approaching a mature sporidium. The dotted half circle represents the hemispherical globule of water that comes into contact with the sporidium. B. Inside of a Petri-dish cover showing the method of mounting telial material of *Puccinia graminis* for inoculation purposes. Reduced.

another so that the sporidium and the end of the tube were separated by a very small distance. A light pressure on the rubber tubing caused the water in the tube to bulge out in hemispherical form, just far enough to establish contact with the sporidium. On release of the pressure, the water went back into the tube, carrying with it the sporidium.

The mount was drawn back as quickly as possible by a reverse movement of the mechanical stage to prevent contamination of the tube by other sporidia. The tube was removed, and, by a light pressure on the rubber tubing, a droplet of water containing the sporidium was deposited on a barberry leaf. The plant was then placed in an incubation chamber for 48 hours. At the end of that period, it was set on a bench in the greenhouse. After each inoculation, the capillary tube was sterilized in boiling water.

If a bisporidial inoculation was to be made, the process was repeated, and the two sporidia were deposited on the leaf in the same drop.

Generally, the second and third sporidia on a basidium mature first, and at about the same time; later, the one towards the tip; and, finally, the one situated nearest the teliospore. The latter one not infrequently aborts and collapses before it reaches maturity.

The comparative failure of this method of inoculation seemed to be due chiefly to physical causes. The waxy cuticle of a barberry leaf has little or no affinity for a droplet of water, so that, when one was placed on a leaf, it remained in almost spherical form, as if placed on an oily surface. It seems probable that the sporidium was held in the drop of water and

either did not germinate or, if it did germinate, its germ tube had little opportunity of establishing contact with the leaf.

(2) *Method B*

This method was much simpler. Petri dishes containing sufficient water to cover the bottoms were used as germination chambers for the teliospores of *Puccinia graminis* and *P. helianthi.*

Inoculations with *Puccinia helianthi* were made as follows. Pieces of rusted sunflower leaves were soaked in water for an hour. Some Petri-dish covers were then lined with thin pads of filter-paper. The leaves were placed in the Petri-dish covers so that their upper sides were in contact with the filter-paper, while their lower sides, which bore the telia, were turned upwards and exposed to the air. The moisture caused the filter-paper to adhere to the covers, and the leaves, in turn, to the filter-paper; so that, when the covers were set on their respective dishes, both the filter-paper and the pieces of leaves remained in position.

If inoculations with *Puccinia graminis* were to be made, the procedure was similar, except that the rusted pieces of culms were supported by short pieces of twine (Fig. 4, B), the ends of which were made fast to the covers by means of sealing wax.

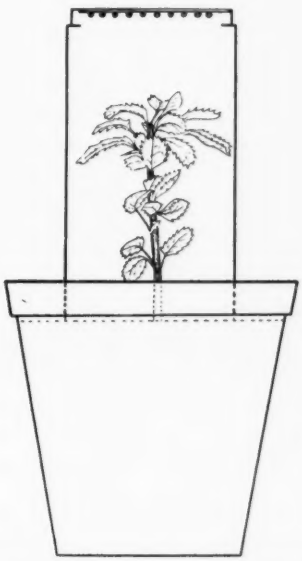

FIG. 5. Semi-diagrammatic drawing to show a barberry plant being inoculated by method B (Fig. 4). The small circles at the top represent short pieces of straw bearing rust sori. The pieces of straw are attached to the inner side of a Petri-dish cover which rests on the end of the hollow cylinder surroundings the plant.

With both *Puccinia graminis* and *P. helianthi*, the same method of inoculation was employed. The plant to be inoculated was first covered with a fine film of water. Over it was inverted a hollow cylinder (Fig. 5), at the upper end of which was held a Petri-dish cover bearing the leaves on which the sporidia were developing. The cover was kept continually in motion by a circular movement of the hand. The cylinder prevented air currents from carrying the sporidia away from the plant, and the movement of the cover insured an even distribution of the sporidia over the leaf surface. After inoculation, the plant was placed in an incubation chamber for 48 hours. At the end of that period, it was placed on a bench in the greenhouse.

As a rule, the sporidia settled on a leaf at some distance apart; but, sometimes, two of them settled close together. The sporidia were not actually seen on the leaves after they had settled, but their location was inferred from the position of the infections to which they gave rise (Fig. 6).

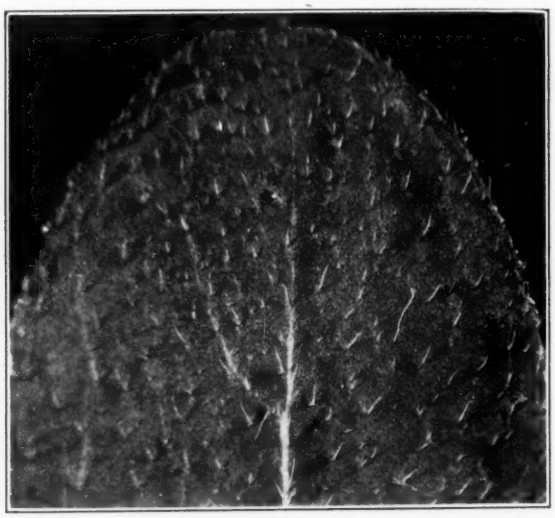

FIG. 6. Under side of a portion of a sunflower leaf showing two neighboring monosporidial pustules shortly after they appeared. These two simple pustules will soon coalesce to form a compound pustule. ×2.5.

The time required for each inoculation varied inversely with the number of sporidia which were being produced. An index of this number was obtained just before the inoculations began. A glass slide was placed in each Petri dish directly below the telia and was left there for 10 minutes. Thereafter, each slide was examined under the microscope, the number of sporidia that had settled on it was noted, and then the time necessary for

an inoculation was estimated and marked on the outer surface of the cover of the Petri dish. If the sporidia were relatively numerous, the time during which it was necessary to expose the leaves to the falling sporidia was short; if relatively scarce, the time was longer. Usually the time varied from 2 to 5 minutes.

EXPERIMENTAL RESULTS IN 1926

During the year 1926, inoculations were made exclusively by Method A. By it two monosporidial pustules of *Puccinia graminis* were obtained on barberry leaves. One of these appeared on a young leaf on July 9. It developed readily and eventually attained a diameter of 6 millimeters. The other one occurred on a comparatively old leaf on August 3. It grew less rapidly and scarcely attained half the diameter of the first one. Both pustules, however, developed pycnia which exuded nectar containing numerous pycnispores. But, although both pustules remained healthy for 5 weeks, neither of them produced aecia. A cytological examination of each pustule, when 5 weeks old, showed that its mycelium was still in the haploid condition.

Three of the bisporidial inoculations by this method were successful. On one of the leaves inoculated on August 5, two neighboring infections occurred. These appeared first as tiny, pale yellow pustules, approximately 2 mm. apart. About 6 days later, the two pustules coalesced. Five days after coalescence, aecia began to appear in the compound pustule thus formed. On August 17, two other barberry leaves were successfully inoculated. Two pustules, about 2 mm. apart, developed on each leaf and coalesced 7 to 8 days later. Aecia appeared in one of the compound pustules so formed within 6 days, but none appeared in the other one within that time or thereafter.

These results, although few in number, were of considerable significance, for they at least indicated that the mycelia of the pustules, and consequently the sporidia from which the mycelia originated, were of two sexes and that in all probability *Puccinia graminis* is heterothallic. They also were indicative of the behavior that might be expected of pustules in further experimentation.

EXPERIMENTAL RESULTS IN 1927

So far in the investigation, *Puccinia graminis* was employed; but, as the teliospores of this rust begin to germinate rather late in the spring—towards the end of April in Canada—it was decided to select another rust, one in which teliospore germination occurs earlier, for the preliminary investigational work of 1927. *P. helianthi* was chosen. Its teliospores were known to germinate earlier in the spring than those of *P. graminis*, and an

abundance of telial material was easily procurable. Moreover, as the writer wished to try inoculation Method B, this rust seemed well adapted to this purpose. In order to have telial material available, a liberal supply of rusted sunflower leaves was collected late in the autumn of 1926 and stored in the basement of the laboratory.

Towards the end of February, 1927, the teliospores of *Puccinia helianthi* began to germinate. Sunflower seedlings were inoculated by sowing sporidia sparsely over the leaves, as already described. Infections became manifest about 8 days after inoculation as tiny red pustules on the leaves. Very frequently the pustules were isolated, often only one on a leaf, or two or three rather widely separated from one another on the same leaf. Less frequently, two pustules arose relatively close together, from 1 to 4 mm. apart, and later coalesced to form a compound pustule. Occasionally three or four pustules occurred in a cluster, but these were discarded forthwith.

As soon as the pustules became visible, the position of the pustule, or pustules, on each leaf was mapped on a label which was then attached to the leaf. In this way it was possible to determine, when the pustules became older, which pustules were simple in origin (monosporidial) and which were compound in origin (bisporidial). If the distance between two neighboring pustules was greater than 4 mm. each one was considered as a simple pustule. Usually two neighboring pustules were sufficiently far apart when they first appeared to be readily distinguishable from each other (Fig. 11); but, occasionally, it was difficult to decide whether or not the infection giving rise to a pustule was of monosporidial or of bisporidial origin. In these cases, which fortunately were not numerous, the pustules were considered as monosporidial in origin.

In the course of the investigation, many sunflower seedlings were inoculated and, as a result of these inoculations, a large number of pustules were made available for study. On account of the uniformity of technique and the similarity of results in all the earlier experiments, it is unnnecessary to describe the experiments in consecutive order or to record separately the data for each one. It will suffice to give a general descriptive statement concerning the development of both simple and compound pustules, and a summary of the experimental results obtained with each type.

Evidence of heterothallism in Puccinia helianthi. The simple (monosporidial) pustules of *Puccinia helianthi* developed vigorously. A few of them finally attained a diameter of 12 mm., but usually the diameter was much less, from 6 to 8 mm. Pycnia developed plentifully on the upper side of all the pustules and less numerously (Fig. 7), or not at all, on the under side. When they were present on the under side, they were gen-

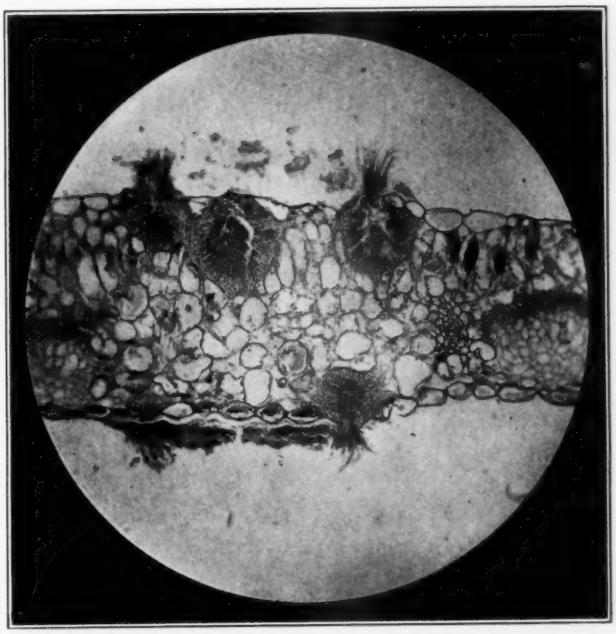

FIG. 7. Section through a part of a monosporidial pustule of *Puccinia graminis* on a barberry leaf showing pycnia present on both sides of the pustule. Magnification, 100. From a photomicrograph by W. F. Hanna.

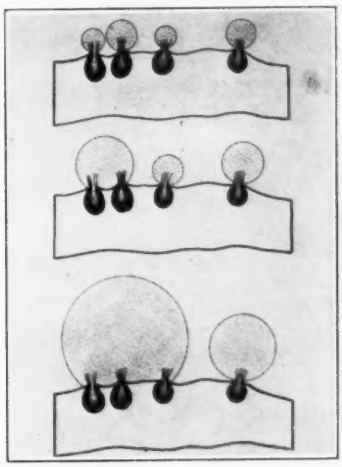

FIG. 8. Diagrammatic representation of a vertical section through a barberry leaf to show how the globules of nectar produced by the pycnia enlarge and finally fuse to form a layer of nectar over the whole surface of the pustule. The small dots in the globules represent pycniospores.

erally most numerous in the peripheral region of the older pustules, many of which were beginning to die at the center. Nectar, containing numerous pycniospores, was exuded by the pycnia of both sides of the pustules. If the pycnia were relatively close to one another, as on the upper side of the pustules, the globules of nectar, as they grew larger, came into contact and fused, so that eventually the whole surface of the pustule was covered with a layer of nectar. The process is illustrated diagrammatically in figure 8.

The majority of the simple pustules never produced aecia (Fig. 9, A), although some of them remained alive for upwards of 5 weeks. Aecia, however, appeared in a minority of them. Out of a total of 2,153 simple pustules, 1,641 went through the whole course of their development without producing aecia. The remainder, 512 in all, at one time or another developed aecia.

No regularity or periodicity marked the appearance of aecia in the latter pustules. Aecia might appear in one of them at any time after it was

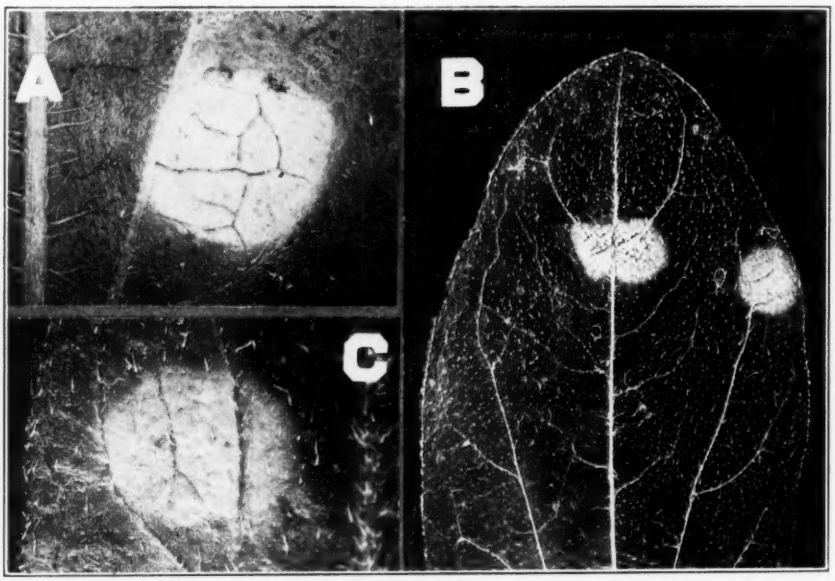

FIG. 9. A. Under side of a portion of a sunflower leaf showing a monosporidial pustule of *Puccinia helianthi* which has not produced aecia. A few pycnia (dark specks) are seen scattered over the surface of the pustule. Photographed 33 days after inoculation. ×5. B. Under side of a sunflower leaf showing a compound pustule of *P. helianthi* astride the midrib, with aecia, and a monosporidial pustule without aecia at the right-hand edge. Photographed 16 days after inoculation. ×1.75. C. Under side of a portion of a sunflower leaf showing a compound pustule of *P. helianthi* without aecia. Photographed 24 days after inoculation. ×4.

10 or 12 days old up to the time of its death, 3 or 4 weeks later. The sudden and spontaneous change from the haploid to the diploid condition took place without any apparent cause, thereby simulating a parallel phenomenon in the Hymenomycetes.

Each compound pustule came into being as a result of the coalescence of two neighboring simple pustules. The time necessary for two such pustules to accomplish coalescence depended largely on the distance they were separated. If the two pustules were about 1 mm. apart, they coalesced early, within 2 or 3 days after they appeared; if they were more widely separated, they coalesced later, possibly from 8 to 10 days after they appeared; but, if they were more than 4 mm. apart, they rarely coalesced. Prior to coalescence, each of the two pustules developed independently as a simple pustule. Following coalescence, one of two things happened. Either aecia appeared in the compound pustules thus formed within from 5 to 6 days (Fig. 9, B) or they did not appear (Fig. 9, C). Of the 246 compound pustules of *Puccinia helianthi* that came under observation, 108 developed aecia within from 5 to 6 days, while in the 138 others no aecia appeared within that time. However, some of these 138 pustules produced aecia at one time or another afterwards, so that, by the time all of the 246 pustules had died, those that had developed aecia had increased from 108 to 145, leaving 101 that had failed to produce aecia.

Evidence of heterothallism in Puccinia graminis. Experiments similar to these just described were made with *Puccinia graminis,* and similar results were obtained. As the barberry plants that were suitable for inoculation purposes were somewhat limited in number, the pustules available for observation were fewer than those of *P. helianthi.* Both simple and compound pustules appeared. Each pustule arose as a tiny pale yellow spot on the leaf. The difficulty in determining whether certain pustules were of monosporidial or of bisporidial origin was again encountered. Possibly, owing to the greater resistance offered by the leaf tissue of the barberry to the radial advance of the mycelium, the pustules of *P. graminis* did not grow so rapidly, or become so large, as those of *P. helianthi.* Very few of them exceeded 5 mm. in diameter. On account of this rather restricted growth, coalescence of pustules 4 mm. apart rarely occurred. On the other hand, the pustules of *P. graminis* produced nectar more copiously than did the pustules of *P. helianthi,* and they possessed greater longevity. The sunflower leaves aged more quickly than the barberry leaves, and the earlier aging of the *P. helianthi* pustules seemed to be attributable more to the decreased vigor of the leaves than to any lack of vitality on the part of the organism.

The data concerning the simple pustules of *Puccinia graminis* may be summarized as follows. Ten days after the first appearance of the pustules,

the number of simple pustules in which aecia had developed and the number in which aecia had not developed were recorded. Out of a total of 174 simple pustules on which observations were made, 11 produced aecia within that time, while 163 were without aecia (Fig. 10, A). From time to time one or another of these pustules developed aecia, so that, when practically all the pustules had died, it was found that altogether 37 of the simple pustules had produced aecia and 117 had failed to produce aecia. The apparently spontaneous change from a haploid to a diploid condition of the mycelium, as evidenced by the production of aecia in a certain number of the pustules of monosporidial origin, thus was observed in *P. graminis*, just as in *P. helianthi*.

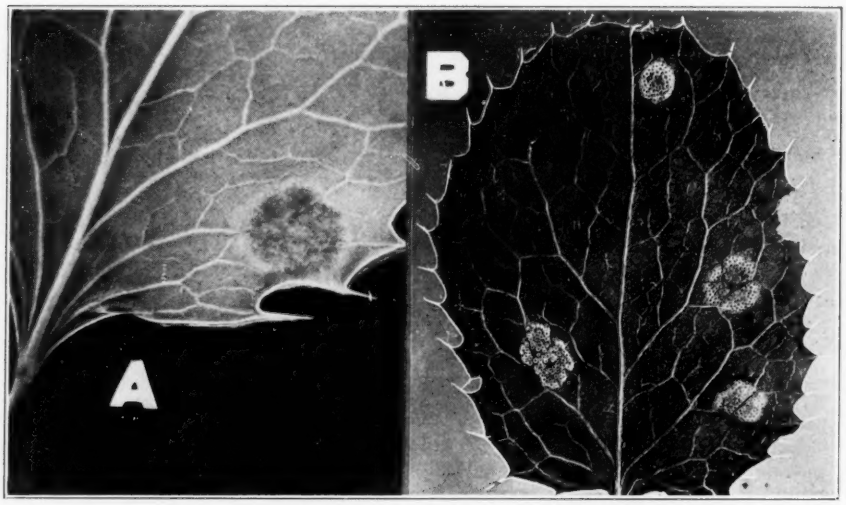

FIG. 10. A. Under side of a portion of a barberry leaf showing a monosporidial pustule of *Puccinia graminis* without aecia. Photographed 23 days after inoculation. ×3. B. Under side of a barberry leaf showing one compound pustule of *P. graminis* with aecia on the left of the midrib and three monosporidial pustules without aecia on the right of the midrib. For the peculiar pocked appearance of the three right-hand pustules *vide* the text. Photographed 20 days after inoculation. ×2.

In the compound pustules 5 or 6 days after coalescence had been effected, a record was made of those pustules that had produced aecia and of those that had not produced aecia. In a few of these pustules, coalescence was not thoroughly accomplished at that time. The number in which aecia were present was 24 and the number in which aecia were not present was 35. At the termination of the experiments, when most of the pustules had died, the proportion was 36 with aecia and 23 without aecia.

Figure 10, B, shows the under side of a barberry leaf, photographed 20 days after it was inoculated. On the left-hand side of the midrib appears a

compound pustule bearing aecia. On the right-hand side are three simple pustules in which no aecia developed. The pocked appearance of these three pustules is due to the formation of wefts of haploid hyphae which develop in simple pustules just beneath the epidermis and simulate in general contour a young aecium.

Mention was made of these structures by Olive (59) in 1911. He believed them to be sterile aecia. They were observed by the writer (15) in 1928. Hanna (29), in 1929, referred to them as being "evidently haploid rudiments of aecial cups waiting to be stimulated into further developmental activity." In the same year, Miss Allen (1) stated that they resemble aecia but "consist of haploid mycelium only." As this paper is not primarily concerned with the cytological aspect of the problem, no further reference will be made to these haploid hyphal wefts.

The results thus far enumerated indicate that *Puccinia graminis* and *P. helianthi* are heterothallic and that the mycelium of an individual pustule is either (+) or (−) in its sexual nature, so that, when a (+) pustule and a (−) pustule coalesce, a sexual interaction takes place between the two mycelia which results in the production of aecia; but that, when two pustules of the same sex coalesce, *i.e.*, a (+) pustule with another (+) pustule or a (−) pustule with another (−) pustule, no sexual interaction occurs and no aecia are produced.

Although heterothallism accounts for the behavior of the majority of the simple compound pustules in *Puccinia graminis* and *P. helianthi,* it gives no explanation of the apparently spontaneous change from the haploid to the diploid condition in simple pustules, or in compound pustules which have long remained sterile. A partial explanation, at least, of this behavior was brought to light when the function of the pycnia was discovered.

Before treating of the individual experiments which deal with the function of the pycnia, it might be pointed out that in each experiment the treated pustules and those kept as a control were of the same age and of comparable vigor. All of the pustules were of monosporidial origin: they bore numerous pycnia, but aecia were entirely absent from them. At the beginning of each experiment, the minimum age of the *Puccinia graminis* pustules was 16 days and of the *P. helianthi* pustules, 14 days. Wherever the experimental technique demanded it, the instruments used were thoroughly sterilized in an alcohol flame.

The effect of mixing nectar. In one experiment with *Puccinia helianthi,* the nectar of 184 simple pustules was intermixed by means of a small scalpel, so that the nectar of each pustule was distributed over the surface of several other pustules. This procedure may have caused a slight irritation of the upper surfaces of these pustules; and, therefore, to procure comparable conditions in 174 other similar pustules, which served as a control,

the nectar of each of these pustules was stirred separately with the scalpel but not mixed with any other nectar.

Five days after the beginning of the experiment, the condition of the pustules was as follows: of the 184 pustules in which the nectar had been mixed, 176 had produced aecia, 4 no aecia, and 4 had wilted and died through leaf injury; of the 174 pustules in which the nectar had been stirred but not mixed, only 20 had produced aecia, while 154 were entirely free from aecia (*cf.* Fig. 11).

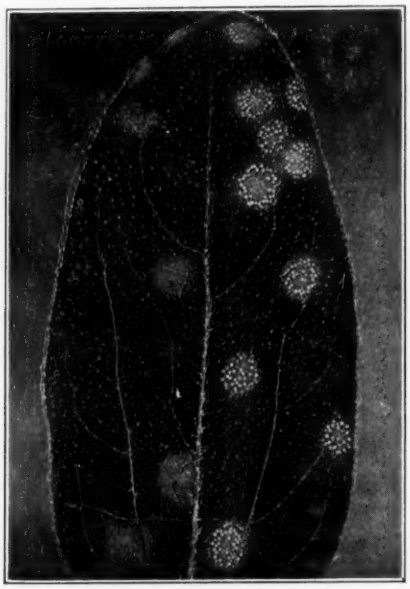

FIG. 11. Under side of a sunflower leaf showing monosporidial pustules of *Puccinia helianthi* on either side of the midrib. Twenty days after inoculation, all the pustules were free from aecia. At that time, the nectar of the pustules on the right of the midrib was well mixed; while, as a control, the nectar of the pustules on the left of the midrib was stirred separately but not mixed. The right-hand pustules have all developed aecia, but the left-hand pustules show no aecia whatsoever. Photographed 6 days after the mixing was done. ×1.5.

An experiment similar to that just described was made with *Puccinia graminis*. The nectar on the upper surface of 116 simple pustules was intermixed; while, as a control, the nectar of 85 other similar pustules was stirred separately but not mixed with any other nectar.

Six days after the experiment had begun, the condition of the pustules was as follows: of the 116 pustules in which the nectar was mixed, 102 had produced aecia and 14 no aecia; whereas, of the 85 pustules in which the nectar was stirred but not mixed, 17 had produced aecia, while 68 were free from aecia (*cf.* Fig. 12, A).

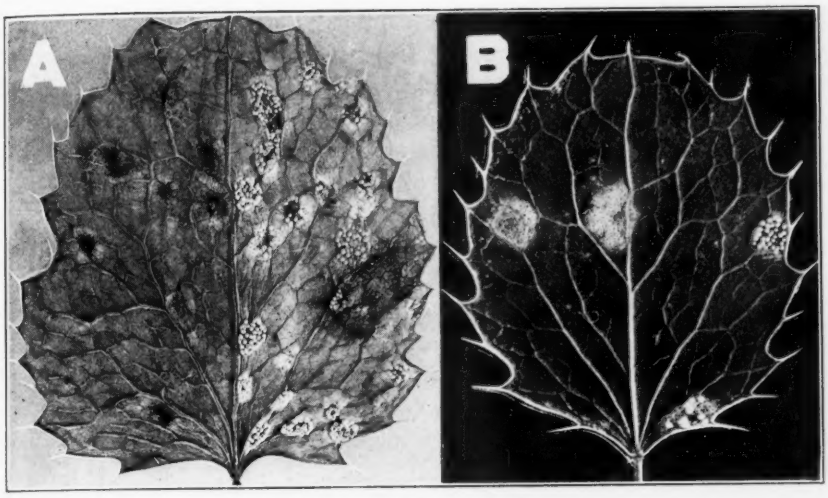

FIG. 12. A. Under side of a barberry leaf showing monosporidial pustules of *Puccinia graminis* on either side of the midrib. Thirteen days after inoculation, one pustule, on the right of the midrib, produced aecia. All of the others were free from aecia 17 days after inoculation. At that time, the nectar of the pustules on the right of the midrib was well mixed; while, as a control, the nectar of the pustules on the left was stirred separately but not mixed. All the pustules on the right-hand side of the leaf have developed aecia, but the left-hand pustules show no aecia whatsoever. Photographed 9 days after the mixing was done. ×2. B. Under side of a barberry showing 4 monosporidial pustules of *P. graminis*. Sixteen days after inoculation, the pustules were free from aecia. At that time nectar heated for 3 hours at 70° C. was applied to the upper surface of the 2 pustules on the left of the midrib, while unheated nectar was applied to the upper surface of the 2 pustules on the right of the midrib. The right-hand pustules alone have developed aecia. Photographed 8 days after the nectar was applied. ×1.5.

The rôle played by flies. Proof that flies mix the nectar of separate monosporidial pustules and so cause the mycelia of the pustules to change from the haploid to the diploid phase, as shown by the appearance of aecia, was obtained in an experiment with *Puccinia helianthi*.

Fifteen to 20 flies were enclosed in a large screen-wire cage with 12 pots of sunflower seedlings on the foliage leaves of which there were 98 simple pustules bearing pycnia but no aecia. As a control, 159 similar pustules on the foliage leaves of sunflower seedlings were protected by a screen-wire cage from the visits of flies.

Eight days after the beginning of the experiment, 96 of the 98 pustules to which flies had access had produced aecia and only 2 no aecia; whereas only 5 of the 159 pustules to which flies had not had access had produced aecia.

It seems very probable that many other insects are active agents in bringing about the transfer of nectar from one pustule to another. Rathay (63) states that he observed 135 different species of insects, belonging to the Coleoptera, Hymenoptera, Hemiptera, and Diptera, visiting pustules of two Gymnosporangium species and also pustules of the common cereal rusts. Shear (68), Grove (25), Meineke (48), and Spaulding (70) also have pointed out that insects are attracted by the nectar.

That insects visit rust pustules is not surprising. It is known that the pycnial nectar of many rusts is sweetish to the taste and that in some rusts the pycnia have a distinct odor. Rathay (63), by chemical tests, showed that the nectar of *Gymnosporangium sabinae* (Dicks.) Wint. contained dextrose and levulose. The odor of the pycnia of certain rusts has been mentioned by Persoon (60), Tulasne (80), Léveillé (39), and Rathay (63). Plowright (61) pointed out that these odors were possibly to attract insects, mimicking as they do the perfume of flowers. No odor has been detected by the writer in *Puccinia graminis* and *P. helianthi,* but he has found that the nectar of the former is distinctly sweetish to the taste.

Effect of heating the nectar. By another experiment it was found that heating the nectar of *Puccinia graminis* or of *P. helianthi* to a temperature of 70° C. for 3 hours to kill the pycniospores rendered the nectar ineffective in inducing the production of aecia when it was applied to the pycnia of individual simple pustules.

Nectar was collected from approximately 50 pustules of *Puccinia graminis* by means of short capillary tubes. One tube was used for each pustule. The nectar was then deposited in a single drop, mixed thoroughly, and sucked up into 2 fine glass tubes, each tube taking up approximately the same amount of nectar. One end of each tube was stopped with sealing wax and the other by a plug of cotton wool. One of the tubes was placed in an oven and kept for 3 hours at 70° C.; the other was kept at room temperature for the same length of time. The heated nectar was then allowed to cool to room temperature, and a small quantity of it was then deposited on each of 25 simple pustules. The droplet on each pustule was mixed thoroughly with the nectar of the pustule to which it was applied. The nonheated nectar was similarly distributed among 20 other similar pustules of *P. graminis.*

Six days later, the condition of the pustules was as follows: of the 25 pustules to which the heated nectar was applied, only 1 developed aecia, while 24 were entirely free from aecia; of the 20 pustules to which nonheated nectar was applied, 17 developed aecia, whereas only 3 were free from aecia.

Figure 12, B, illustrates the results. The pustules on the left of the midrib, to which heated nectar was applied, have produced no aecia; those

to the right of the midrib, to which nonheated nectar was applied, have produced aecia.

A similar experiment was made with *Puccinia helianthi.* Heated nectar was added to 27 simple pustules and nonheated nectar to 25 other similar pustules. At the end of 5 days, aecia were completely absent in 23 of the 27 pustules to which the heated nectar was added, 4 only having formed aecia; while of the 25 pustules to which the nonheated nectar was added, all produced aecia.

These two experiments indicate that the pycniospores are the effective agents in inducing the formation of aecia, and not the nectar. The evidence, however, is not absolute. It is recognized that if the agent were an enzyme in the nectar the heating might destroy its activating property.

Sex of pycniospores. Another experiment was designed to show whether or not the pycniospores of *Puccinia graminis* are of two kinds, (+) and (−). The nectar of one monosporidial pustule of *P. graminis* was drawn off by means of a capillary tube and divided into several drops and then the drops were applied singly to the pycnia of as many pustules as there were drops. The nectar of other simple pustules was divided in like manner and distributed among other pustules. Altogether, 74 individual pustules were thus treated. Six days later, 30 of the 74 pustules had developed aecia, while 44 were free from aecia. As a control, 26 similar pustules were left untreated. Aecia appeared in only one of these.

Figure 13, A, 22 is illustrative of the results. Nectar from one pustule was applied to each one of the four pustules. Aecia arose in two of them but not in the other two. Usually, however, among pustules on the same leaf, the ratio of those with aecia to those without aecia was less evenly balanced.

The experiment just described was repeated with *Puccinia helianthi.* Five days after the drops of nectar were applied, 15 of the 48 pustules treated had developed aecia, whereas 33 of them were without aecia. In a control of 66 similar pustules, only 5 produced aecia within that time.

Theoretically, if each basidium bears two (+) sporidia and two (−) sporidia, the number of pustules with aecia and the number without aecia should be equal in each experiment. The ratio obtained with the pustules of *Puccinia graminis* is possibly as near the theoretical ratio as could be expected with such a small number of pustules. With the pustules of *P. helianthi,* there is a much greater divergence, yet not so great as appears in the control. The rather indifferent ratio given by the pustules of *P. helianthi* is very probably attributable, in some way or other, to the meager quantity of nectar that was being produced by pustules at the time the experiment was performed.

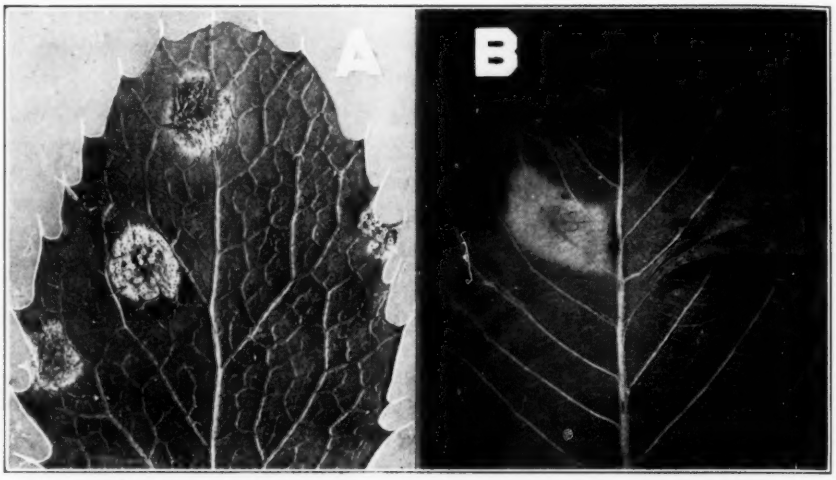

FIG. 13. A. Under side of a barberry leaf showing 4 monosporidial pustules of *Puccinia graminis*. The nectar from a monosporidial pustule on another leaf was divided into 4 small drops and added to the 4 pustules, 1 drop to a pustule, when they were 17 days old. Within 5 days aecia arose in 2 of them but not in the other 2. The photograph was taken 10 days after the nectar was added. ×2. B. Under side of a leaf of *Amelanchier alnifolia* showing a pustule of a *Gymnosporangium* sp. (*corniculans?*) which was 6 weeks old at the tin e the photograph was taken. The pustule failed to develop aecia. ×2.5.

Aecia in monosporidial pustules. It will be observed that in all the experiments so far discussed aecia developed apparently in a spontaneous manner in at least some of the monosporidial pustules. When the effect of mixing the nectar of pustules of opposite sex was discovered, it was thought that possibly mixing of nectar had taken place by some unknown means and that, under carefully controlled conditions, few or none of the pustules would develop aecia. It is to be noted that in the earlier experiments: (1) the pustules were examined frequently, and there was the possibility that, in the repeated handling of the leaves bearing pustules, nectar was transferred from one pustule to another; and that (2) no protection was afforded the pustules from the visits of flies or other insects that might by chance have been in the greenhouse.

A more critical experiment than any of those already recorded was devised with a view to preventing the accidental intermixing of nectar.

Sunflower seedlings were inoculated in the greenhouse by allowing sporidia of *Puccinia helianthi* to fall sparsely onto the upper surface of the first two foliage leaves when they were about one inch in length. Usually, about six seedlings grew in a pot; but, whenever the minute pustules appeared, each plant showing infection was planted by itself in a separate pot

and covered with a screen-wire cage. By keeping each plant in an individual cage, the opportunity for the transfer of nectar from one pustule to another was reduced to a minimum. All uninfected leaves were removed, and no new ones were allowed to develop.

Only pustules that were entirely free from aecia at 17 days of age were selected for observation. Of these there were 228, distributed as follows: 93 pustules were borne singly on as many plants; the remaining 135 were borne on 59 other plants all of which bore at least 2 pustules, usually 1 on each leaf, but a small number of the plants bore 3 pustules, and a very few, 4 pustules.

Not all of the pustules persisted for the same length of time. Some of them died when about 4 weeks old and most of them before they were 6 weeks old, but a few were still living and exuding nectar in their peripheral region when 7 weeks old—when the final data were recorded. Within the 7 weeks, aecia developed in 11 of the 228 pustules. Of the 93 pustules, each of which was borne on a separate plant, only 2 produced aecia. Of the 135 pustules, of which 2 or more were borne on individual plants, 9 produced aecia. Altogether 217, or 95 per cent, remained free from aecia during the whole course of the experiment.

From the time the pustules first appeared, daily inspections were made of the plants in order to destroy any insects that might have gained access to them. White flies (*Aleyrodes*) and thrips (*Heliothrips*) were the only ones discovered, and then only infrequently. The former were rarely found in contact with the pustules, but the latter seemed to be attracted by the nectar and fed upon it in preference to the leaf tissue. Consequently, in spite of the precautions taken, there was the possibility that thrips carried the nectar of one pustule to the pycnia of another, especially on leaves that bore two or more pustules. This assumption is supported by the fact that nine of the pustules that produced aecia occurred on leaves bearing two or more pustules, and only two on plants bearing but a single pustule.

If, in the experiment just described, there had been an absolute exclusion of insects and a complete absence of any means by which the transfer of nectar could have taken place, it is possible that no aecia whatever would have developed in any of the pustules. At present, it is impossible to say that aecia never develop spontaneously in simple pustules of *Puccinia helianthi;* but it has been clearly shown that, under the conditions of the experiment, the percentage of pustules that actually produced aecia was extremely small, not more than 5 per cent. The experiment was not repeated with pustules of *P. graminis,* but later observations showed that, where monosporidial pustules of this rust were protected from the visits of insects, very few of them developed aecia.

Occurrence under natural conditions of pycnia unaccompanied by aecia. So far as the writer is aware, it is not recorded that pustules of monosporidial origin occur in nature, although undoubtedly such pustules have been seen by other observers and mistaken by them for young pustules in which aecia had not yet developed. Observations made during the summer of 1927 and 1928 have revealed that, in nature, pycnia are frequently unaccompanied by aecia in the following rusts: *Puccinia graminis* on *Berberis vulgaris* var. *purpurea; P. coronata* on *Rhamnus cathartica* L.; *P. Pringsheimiana* on *Ribes grossularia* L. (cultivated and wild); and a *Gymnosporangium* sp. (possibly *corniculans* Kern.) on *Amelanchier alnifolia* Nuttall (Fig. 13, B).

Leaves of these hosts bearing young and apparently monosporidial pustules were marked by means of a small tag as soon as the pustules were noticed. When the pustules were about 14 or 15 days old, those showing no signs of aecia were selected for further observation. It was thought that pustules of this age could not include any compound pustules of two mycelia of opposite sex, for compound pustules of this type would have produced aecia when several days younger.

At intervals during the next 3 or 4 weeks, the tagged pustules were examined. Within that time, some of the pustules produced aecia, but others did not. A few of the pustules persisted for a week or more longer, but none of these bore aecia. The results for 1927 are summarized in table 1.

TABLE 1.—*Summary of observations made in 1927 on the occurrence in nature of aecia in monosporidial pustules*

Name of rust	Period of observation (in weeks)	Total number of pustules	Pustles at end of period	
			Number with aecia	Number without aecia
Puccinia graminis	4½	50	37	13
P. coronata	2½	61	45	15
P. Pringsheimiana	3	60	16	44
Gymnosporangium sp.	3	60	52	8

It should be noted that none of these pustules were protected in any way from the visits of insects, and there is little doubt that insects did actually transfer nectar from one pustule to another, as insects of various kinds were seen flying about, or crawling over, the leaves of the host plants, and some of them were even observed in contact with the pustules. However, it also should be mentioned that, on account of the excessive precipitation, the pustules were frequently washed, and only very seldom was

there any noticeable amount of nectar available for transfer. Perhaps the reduction in the amount of available nectar accounts for the failure of so many of the pustules to produce aecia, despite the activity of the insects.

During the summer of 1928, young pustules, apparently monosporous in origin, were again marked, but, instead of leaving them exposed, as was done in the previous year, most of the leaves bearing them were covered with one layer of white cheesecloth in order to prevent insects from visiting the pustules. This protection excluded most of the winged insects but was less effective against ants and small spiders.

Not all the pustules marked were found as soon as they appeared. At the time the coverings were applied, some pustules were at least 1 week old; most of them, however, were just appearing or were not more than 2 or 3 days old. Unfortunately, the pustules available for study were much less numerous this year than in the previous year. There were 20 pustules of *Puccinia graminis* on *Berberis vulgaris* var. *purpurea*, 2 pustules of *P. coronata* on *Rhamnus cathartica*, and 51 pustules of *P. Pringsheimiana* on *Ribes grossularia*. No pustules of the *Gymnosporangium* sp. occurred on *Amelanchier alnifolia*.

About one-third of the pustules on Ribes were left unprotected to serve as a check for the protected ones and for comparison with the data collected in 1927. The results for 1928 are summarized in table 2.

TABLE 2.—*Summary of observations made in 1928 on the occurrence in nature of aecia in monosporidial pustules*

Name of rust	Covered or uncovered	Period of observation (in weeks)	Total number of pustules	Pustules at end of period	
				Number with aecia	Number without aecia
Puccinia graminis	Covered	3	20	4	16
P. coronata	Covered	3	2	0	2
P. Pringsheimiana	Covered	3	33	6	27
P. Pringsheimiana	Uncovered	3	18	6	12

It will be seen from tables 1 and 2 that: (1) a much smaller percentage of the covered pustules in 1928 produced aecia than the uncovered ones in 1927; and (2) that, in 1928, less than 20 per cent of the covered pustules of *Puccinia Pringsheimiana* developed aecia, as compared with 50 per cent of those that were not covered. Apparently, by preventing the free access of insects to the pustules, the opportunity for transferring the nectar of one pustule to another was reduced, and consequently the number of simple pustules which remained free from aecia was augmented. It is not probable that the cheesecloth covering intercepted sufficient sunlight to

inhibit, or retard materially, the formation of aecia. This supposition is supported by the fact that aecia formed in a small number of the covered pustules. The development of aecia in these pustules was very probably induced through the transfer of nectar to them by insects before the coverings were applied.

From the fact that, under natural conditions, monosporidial pustules of *Puccinia coronata*, of *P. Pringsheimiana*, and of the *Gymnosporangium* sp. behave like monosporidial pustules of *P. graminis*, and, from the fact that *P. graminis* has been shown experimentally to be heterothallic, it may be inferred that *P. coronata*, *P. Pringsheimiana*, and the *Gymnosporangium* sp. also are heterothallic.

Aecia limited to one part of a pustule. While the data recorded above were being collected in the field, it was noticed that occasionally aecia were present in only one part of a pustule and not all over it. This phenomenon was observed in pustules of *Puccinia coronata*, *P. Pringsheimiana*, and *P. graminis* (Fig. 14, A). A plausible explanation of this behavior is that nectar from a pustule of opposite sex had been deposited on the upper surface of the pustule but only on the area under which the aecia had developed.

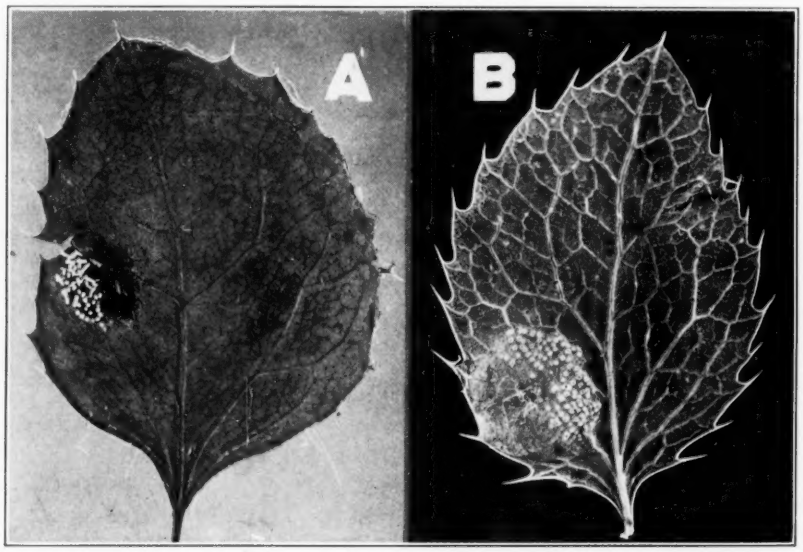

FIG. 14. A. Under side of a barberry leaf showing aecia present on the left-hand side, but not the right-hand side of a pustule of *Puccinia graminis*, which arose from an infection by natural inoculation. ×2. B. Under side of a barberry leaf showing a monosporidial pustule of *Puccinia graminis* to which, when 20 days old, a small quantity of composite nectar was applied to the upper surface of that part in which aecia are now seen, but the other part was left undisturbed. Photographed 7 days after the nectar was applied. ×2.5.

An attempt was made to reproduce experimentally similar results in monosporidal pustules of *Pucciana graminis*. Nectar was collected from about a dozen pustules and deposited on a glass slide in one drop. As (+) and (−) pustules were known to occur in about equal numbers, it was anticipated that in the drop both (+) and (−) pycniospores would be present. The drop was then stirred to distribute the two kinds of pycniospores evenly through it, and a small portion of this composite nectar was spread over one-half of the upper surface of a pustule that was 25 days old. Several other pustules of the same age were similarly treated. Before this nectar was added to a pustule, however, as much as possible of its own nectar was drawn off by means of a capillary tube in order that the nectar which was being added might be more definitely restricted to the area intended for it. Within 5 days, aecia developed in each pustule on the under side of the treated parts but not of the untreated parts (Fig. 14, B).

The experiment was repeated with monosporidial pustules of *Puccinia helianthi*, and similar results were obtained.

In most of the pustules, particularly in those of *Puccinia helianthi*, the aecia remained localized in the manner just described, but, in two or three pustules of *P. graminis*, there appeared to be a tendency for the development of aecia to extend subsequently from the treated half of the pustule, first to the adjacent untreated part, and finally to the remainder of the pustule.

The extension of aecial development just referred to may be explained by assuming that, in the course of a few days after the composite nectar was applied to the pustules, the pycniospores on the treated parts were carried slowly across the untreated parts by a flowing movement of the nectar; for the upper surfaces of the pustules were usually convex or concave, rarely flat; and day by day, as the pustules secreted more nectar, a state of flux was set up, and thus the composite nectar was brought into contact with the pycnia of the untreated parts. In support of this assumption, it may be mentioned that in pustules of *Puccinia helianthi* the amount of nectar exuded is much less than in pustules of *P. graminis* and that in *P. helianthi* aecial development was confined to the parts that received the composite nectar. Another possible explanation is that the mycelium of the treated part may have advanced gradually into the untreated part and there, in some way or other, have caused the development of aecia.

From the above it will be seen that the phenomenon of the occurrence of aecia in one part of a monosporidial pustule, as observed under natural conditions for *Puccinia coronata*, *P. Pringsheimiana*, and *P. graminis*, has been successfully reproduced in the laboratory in experiments made upon *P. graminis* and *P. helianthi*.

Origin of a new physiologic form. During the investigation, the aeciospores from some of the aecia that had been caused to develop by the application of nectar to the pycnia of pustules were handed over to Margaret Newton and Thorvaldur Johnson, of the Dominion Rust Research Laboratory, to inoculate wheat seedlings in order that the physiologic forms represented in these aecia could be identified. It was thought that, possibly in the telial material that produced the sporidia which led to the development of the pustules, two forms of rust might have been present and that, in some of the experiments, hybridization of two forms might have taken place. The form (or forms) present in the parental teliospores was not known; but it was argued that, if a physiologic form hitherto unknown could be identified by sowing the aeciospores on wheat and making cultural studies on the differential wheat varieties, its identification would be partial evidence at least that hybridization had occurred. On the other hand, there was the possibility that, if a new form was found, it might have been present in nature previously without having been discovered. However, as the survey for physiologic forms had been quite thorough in the vicinity of the Agricultural College, Winnipeg, where the telial material was collected, it was not very probable that an existing form had escaped detection. The likelihood was that, if a new form was isolated, it would be the product of a cross, or the result of segregation in a physiological form, a circumstance which, of itself, would be evidence of the hybrid nature of physiologic forms.

From one of the aecial cultures, a new and distinct physiologic form of stem rust was isolated. This form has been designated as No. 57, by Stakman and Levine.

RECENT WORK OF OTHERS ON HYBRIDIZING PHYSIOLOGIC FORMS

Since the investigation here described was carried out, confirmatory evidence that physiologic forms of *Puccinia graminis* do hybridize on the barberry has been obtained by several investigators. The crossing of physiologic forms was effected by the various investigators by transferring pycnial nectar in the manner described by the writer in 1927 and in an earlier part of this paper. Waterhouse (88), in 1929, reported that he had obtained two forms of rust in Australia from crosses made on the barberry. Newton, Johnson, and Brown (57), in 1929, working at Winnipeg, found that, when certain physiologic forms are selfed on the barberry, segregation takes place with the production not only of some old forms but also some new ones. The identification of these old and new forms indicated that the original forms must have been heterozygous for pathogenicity and therefore hybrid in their nature. They found one form that was homozygous. Furthermore, by making reciprocal crosses between forms, they obtained other forms which were apparently the products of direct crosses.

Stakman, Levine, and Cotter (74), in 1929, working at the University of Minnesota, reported successful crosses between *P. graminis tritici* form 36 and *P. graminis agrostidis*, from which they obtained forms that were different, in respect to pathogenicity, from both forms.

DISCUSSION

Experimental evidence from (1) observations on simple (monosporidial) and compound (bisporidial) pustules and from (2) observations on the effect of transferring pycnial nectar has been presented which shows that simple (monosporidial) pustules of *Puccinia graminis* and *P. helianthi* are of two kinds, (+) and (−), and that these two kinds are about equal in numbers. As a deduction, it follows that the mycelia of the pustules, and, therefore, the sporidia from which they originate, are of two different kinds, (+) and (−), and that these two kinds are about equal in numbers. This suggests that segregation of the (+) and (−) factors takes place in the basidium during the nuclear division in the same manner as it was found to take place in *Coprinus Rostrupianus* by Miss D. E. Newton (55) and in *C. radians* by Vandendries (84).

A cytological study of the mycelia of *Puccinia graminis* and *P. helianthi* has not been made; but, from the results obtained by sowing two sporidia of the same species close together on a leaf of the appropriate host, a theoretical explanation of what takes place may, for the present, be offered. It is as follows: When a (+) sporidium and a (−) sporidium are sown close together on a leaf so that the pustules arising from the two infections coalesce, the two monosporous mycelia come into contact, fuse together, and give rise to binucleate aeciospores, each conjugate pair of nuclei formed in the spore bed consisting of a (+) and of (−) nucleus derived from a (+) and a (−) mycelium, respectively. On the other hand, when two sporidia of the same sex, either two (+) or two (−) sporidia, are sown close together on a leaf so that the two pustules arising from the two infections coalesce, the two monosporous mycelia come into contact but do not interact sexually and consequently do not give rise to aeciospores.

The investigation has not finally disposed of the age-long question regarding the real nature of the pycniospores, yet it has produced some tangible evidence from which certain inferences may be drawn. In the first place, it has shown that pycnia develop in every pustule of monosporidial origin and that they all produce pycniospores; secondly, that (+) pycnia produce (+) pycniospores, and (−) pycnia, (−) pycniospores; thirdly, that the application of pycniospore-containing nectar taken from a (+) pustule to the pycnia of a (−) pustule, or *vice versa*, leads to the production of aecia in the pustule to which the nectar is applied; and, lastly, that, when the pycniospores in the nectar are killed by heat, the nectar loses this activating property.

Since pycniospores are produced by the pycnia of every monosporidial pustule, it is clear that, if the pycniospores are male gametes (spermatia), *Puccinia graminis* and *P. helianthi* are not dioecious. In other words, the monosporidial mycelia are not of two kinds: (a) male, bearing spermatia, and (b) female, not bearing spermatia.

Assuming that the pycnia are male conceptacles and that the pycniospores are gametes, it is logical to expect that female counterparts would also be present. Such counterparts, however, have not been discovered.[2] Their absence might be explained by their complete degeneration; but, in the evolutionary development of other fungi, in those forms where degeneration of the sexual organs occurs, it is the male elements that first undergo degeneration. If in the pycnium-producing rust fungi the female structures have entirely disappeared, these fungi occupy a wholly anomalous position among the fungi. Particularly is this true if the pycniospores perform some definite function. The evidence advanced in this paper indicates quite clearly that they do. Complete degeneration has then overtaken the female elements, while the male elements have retained their pristine vigor.

That the pycniospores are not male gametes seems most probable. The writer is inclined to regard the pycniospores as conidia corresponding to the uninucleate oidia of such heterothallic Hymenomycetes as *Coprinus lagopus, C. niveus, Stereopharia semiglobata,* and *Collybia velutipes.* In these species, oidia are produced by both (+) and (−) monosporous mycelia, and cell fusions between (+) and (−) mycelia initiate the diploid phase.

The pycnia may then be regarded, not as spermogonia producing nonfunctional spermatia, but as active organs which develop either (+) or (−) pycniospores and attract flies and other insects by means of which the pycniospores of one sex are carried to the pycnia of another sex. They occur chiefly on the upper side of leaves where they are readily accessible to insects, and they are usually red or yellow, by which means they are made conspicuous. Odors are emitted by the pycnia of some rusts and in many species the nectar is sweetish. These properties, no doubt, are advantageous

[2] This statement is no longer correct, and the opinion here expressed concerning the nature of the pycniospores is no longer tenable. Andrus (2) has found hyphae in *Uromyces appendiculatus* (Pers.) Fries and *U. vignae* Barclay that, in his opinion, are functionally the equivalent of trichogynes. The pycniospores function as spermatia. Recent unpublished results of research carried on by Ruth F. Allen, of the Bureau of Plant Industry, U. S. Department of Agriculture, show that in the gametophytic (haploid) generation of *Puccinia coronata* Corda, *P. graminis* Pers., and *P. triticina* Eriks. certain hyphae grow either to the ventral or to the dorsal surface of the host leaf by pushing into stomata or between epidermal cells, or even by growing through an epidermal cell. Any hypha reaching the surface may serve to receive spermatia and initiate the sporophytic (diploid) generation.

to the species that possess them in that they attract flies and other insects and thus insure that the function of the pycnia is fulfilled.

It has long been remarked that, in those rust fungi which possess them, the pycnia are the first spore-producing organs to appear. Since they play such an important part in changing the haplophase into the diplophase and in inducing the formation of aecia, their appearance on the mycelium in advance of the aecia can be readily understood: pycnia precede aecia because by their action aecia are formed.

Under natural conditions, there are at least two ways in which pustules of monosporidial origin may change from the haploid to the diploid condition: (1) by a (+) sporidium and a (−) sporidium settling on a leaf close together so that they form pustules which coalesce in such a way that the (+) mycelium and the (−) mycelium come into contact directly; and (2) by means of flies and other insects which carry the pycniospore-containing nectar of a (+) pustule to the pycnia of a (−) pustule or, conversely, carry the pycniospore-containing nectar of (−) pustule to the pycnia of a (+) pustule.

Whether or not a spontaneous change from the haploid to the diploid condition occurs in the mycelia must be further investigated. A phenomenon of this kind occurs in certain Hymenomycetes, but, in *Puccinia graminis* and *P. helianthi,* the apparently spontaneous change may be tentatively attributed to the fortuitous intermixing of nectar.

The crossing of two physiologic forms of *Puccinia graminis, P. helianthi,* or of any other heterothallic rust species which behaves like *P. graminis* and *P. helianthi,* may then take place in two ways: (1) by the union, within the tissue of one and the same host plant, of the (+) mycelium of a pustule belonging to one form with the (−) mycelium of a pustule belonging to a different form, or *vice versa;* and (2) by the application of the pycniospore-containing nectar of a (+) pustule of one physiologic form to the pycnia of a (−) pustule of another physiologic form, or by the application of the pycniospore-containing nectar of a (−) pustule of one physiologic form to the pycnia of a (+) pustule of another physiologic form. The second method is much more simple than the first, and it was used by all those investigators who have recently succeeded in crossing physiologic forms of rust.

SUMMARY

(1) A study was made of the sexual behavior of two rusts, *Puccinia graminis and P. helianthi.*

(2) An apparatus is described by which single sporidia can be picked off their sterigmata.

(3) Two methods of inoculation are described which make it possible to obtain monosporidial and bisporidial pustules.

(4) Pycnia developed on the upper side of each and every pustule of *P. graminis* and *P. helianthi*, and to a lesser extent on the lower side, being most abundant there in the peripheral regions of the older pustules.

(5) The pycnia of each and every pustule of *P. graminis* and *P. helianthi* exuded nectar containing numerous pycniospores.

(6) Both *P. graminis* and *P. helianthi* are heterothallic. Some evidence was secured which indicates that *P. coronata*, *P. Pringsheimiana* and a *Gymnosporangium* sp. (*corniculans*?) are also heterothallic.

(7) A large majority of the monosporidial pustules of *P. graminis* and *P. helianthi* failed to produce aecia.

(8) A small minority of the monosporidial pustules of *P. graminis* and of *P. helianthi* produced aecia at some time during the course of their development, possibly owing to a fortuitous mixing of their nectar by handling or through the agency of insects.

(9) Approximately half of the compound (bisporidial) pustules of *P. graminis* and of *P. helianthi* produced aecia, while the remainder produced none.

(10) The monosporidial pustules of *P. graminis* and of *P. helianthi* are of two sexes, (+) and (−). The (+) pustules are about equal in number to the (−) pustules. This fact indicates that, in all probability, the sporidia which are produced by the basidia and to which the pustules owe their origin are divisible into two groups, (+) and (−), which are about equal in number.

(11) Intermixing the nectar of monosporidial pustules of *P. graminis* or of *P. helianthi* induces the formation of aecia in the pustules so treated within from 5 to 6 days.

(12) Flies are active agents in carrying the nectar of one pustule to another and in thus affecting the transfer of pycniospores from (+) pustules to (−) pustules and of (−) pustules to (+) pustules.

(13) Nectar heated to 70° C. for 3 hours to kill the pycniospores does not induce the formation of aecia in the pustules to which it is applied.

(14) Some monosporidial pustules of *P. coronata*, *P. Pringsheimiana*, and a *Gymnosporangium* sp., found in nature, failed to produce aecia, thereby indicating that these species are heterothallic.

(15) Nectar of a (+) pustule applied to the pycnia in one part of a (−) pustule induces the production of aecia in that part to which it is applied, and rarely beyond that part.

(16) The origin of a new physiologic form of *P. graminis* is reported.

(17) Two methods are described by which a cross may be made between two physiological forms of a heterothallic rust which produces pycnia and aecia.

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INVESTIGATIONS ON THE BLACK ROOT OF STRAWBERRIES[1]

FORREST C. STRONG AND MIRIAM C. STRONG

INTRODUCTION

Among the cultivated crops the strawberry has long been considered one in which the disease hazard for the grower is small. Mildew, leaf spot, and a few fruit rots have at one time and another attracted the attention of growers. For the most part these diseases are of minor importance, and no control measures are practiced.

There is, however, a situation in strawberry culture which is a subject of concern to the growers. This consists in the failure of plants newly set out to become established and, often during the picking season, the dying of plants already established. In severe cases this latter condition may be so extensive as to render the plantation unprofitable. Losses in stand of plants in Michigan plantations have been observed to range from 5 per cent to as high as 60 per cent, in some cases, with consequent reduction in crop yields. A typical example is the case of a 1-year-old plantation located in the central part of the State where the grower set in new plants twice in an unsuccessful attempt to secure a full stand (Fig. 1). Many

FIG. 1. A 20-acre plantation near Edmore, Michigan, showing effects of black root.

[1] Journal article 60 (n. s.) from the Agricultural Experiment Station of the Michigan State College, published by permission of the Director. This paper includes, in part, material presented by the senior writer as a thesis in partial fulfillment of the requirements for M. S. degree, M. S. C., June, 1927. The writers acknowledge indebtedness to Dr. E. A. Bessey and Dr. G. H. Coons for suggestions and advice during the course of the investigation.

other similar cases have been observed. Losses of plants in older plantations are particularly prevalent at the time of fruiting.

It is suggested that along with drought injury and winter killing, the disease that is the subject of this article is an important factor in the difficulties encountered in securing and maintaining a stand of strawberries. As the discussion proceeds, it will be seen that we are concerned with a root disease that, by its weakening effect on plants, renders them low in vitality and subject to injury by various factors, such as drought and frost. The conditions to be described will be seen to play an important rôle in the deterioration of strawberry plantations.

THE BLACK-ROOT DISEASE

A strawberry plant affected with black root has a characteristic appearance. The diseased plant shows low vitality, is usually stunted, and has a tendency to dry up at the onset of dry-weather conditions. Such a plant, on being dug up, is found to have a root system in which the roots are all black, corky in texture, and apparently dead. If a diseased plant, not yet in the wilting stage, be examined, there will be seen young white lateral roots pushing out from roots with blackened cortical tissue. On examination of such a root the diseased cortex will be found to peel off readily, leaving the vascular cylinder from which the secondary roots arose still more or less white and apparently functioning. It should be borne in mind that roots dying from age present much the same general appearance as that just described.

The examination of a younger plant affected with black root yields a more clean-cut picture of the disease. Even these younger plants show characteristic evidences of the disease by the production of smaller leaves and fewer main roots, together with the reduction in number of lateral roots. Young plants affected with black root lack vigor, as is manifested by their slow growth and reduced runner production. Some of the main roots of such plants will be rotted their entire length and brown to black. From few to many lesions are to be found on the main roots and many or all of the lateral or secondary roots will either be rotted off or show distinct rotted areas (Fig. 2).

The lesions on the main roots vary in size from 0.5 to 5 cm. in length and usually encircle the root. At first they are reddish brown and later become black. These lesions, in their early stage, exhibit a brown, water-soaked appearance, but very soon become shriveled and shrunken. Very often the rotting involves all of the root tissue, affecting the stele or vascular cylinder, as well as the cortical tissue. Such roots break at the rotted portion. The lesions on the lateral roots resemble in miniature those on the main roots.

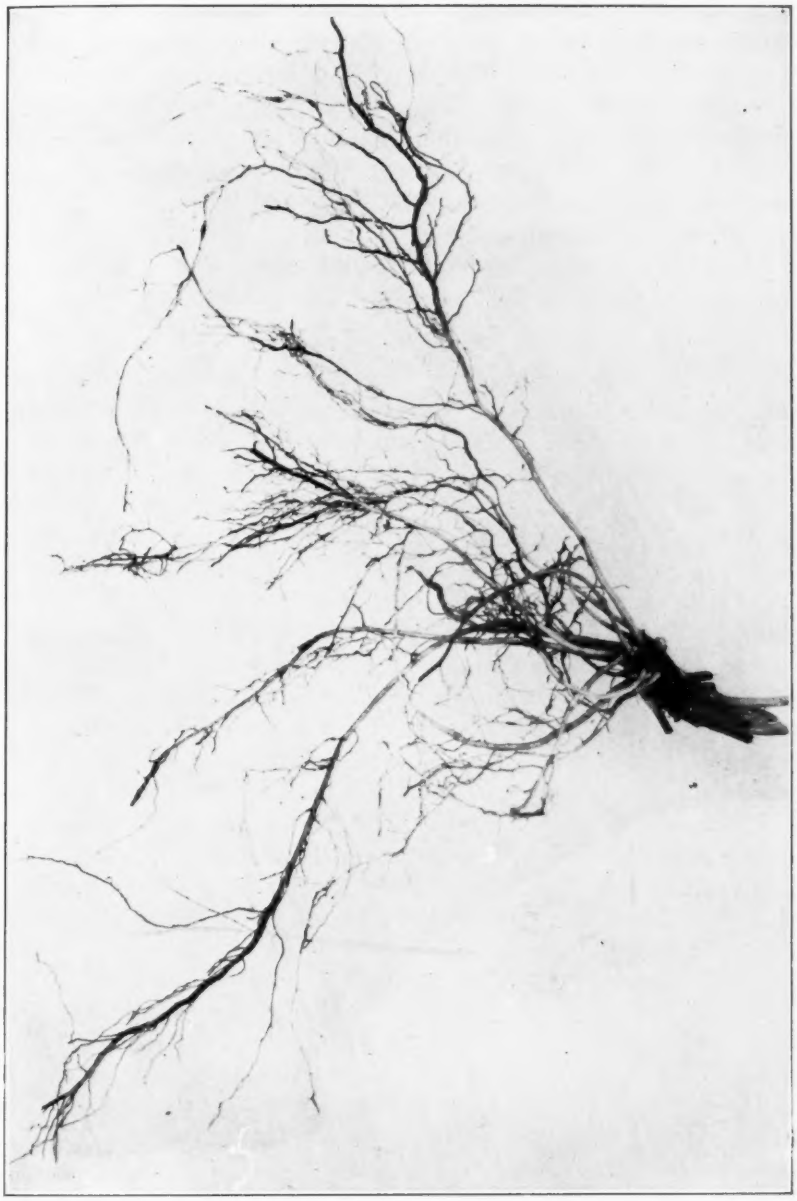

FIG. 2. Young plant showing natural infection.

It is probable that the attack upon the lateral roots is very important in producing the general effects of black root, since the root hairs through which water and plant-food materials enter the plant largely occur on these portions of the root system. The loss of these lateral roots then reduces the efficiency of the root system. This lack of a properly functioning root system is seen in plants affected with black root or root rot during dry periods of summer and especially during the picking season, if it is at all dry. On a hot day the leaves will wilt down and then recover during the night. If the dry weather continues, the plants will die, the berries shriveling and hanging green or partially ripened on the stem. The leaves frequently become purplish or bronzed, and the petioles turn red.

PREVIOUS WORK

Writers on strawberry culture have long referred to black root as a diseased condition of unknown cause. There is little to be gained by referring to these scattered notes, since none of the older writers made more than casual comment on the severity and probable cause.

What may be a typical case of black root was described by Clinton (5) under the heading "Leaf Scorch," but he stated that no fungus appeared to be affecting the roots. Fletcher (8) in 1917 describes "root rot or black root" of strawberry and states that the disease was prevalent in New York, Michigan, and Massachusetts in the years 1902 to 1908. "When the berries are about half grown the plants wilt and turn yellow; the roots are decayed. Most of this trouble is due to winter injury, but a bacterial disease is associated with it in some cases. Poor culture, lack of fertility, the crowding of plants in the row, insufficient mulching, and wet land are favorable for this trouble." Heald (10) in 1920 mentioned the "dying out" of strawberry beds in western Washington and ascribed the trouble to Rhizoctonia. The next year Smith and Horne (16), in California, described a rot of strawberries in which the cortex of the root decayed. No parasite was found and the rot was believed due to water-logging of the soil or sudden drying out of soil moisture. In the summer of 1923 the disease was reported from Mississippi by Neal (11), who wrote: "A root rot of strawberry has been discovered in many parts of the state the present season. The disease causes a distinct wilting of the plants, accompanied by pronounced discoloration of the roots and crown tissue. A species of Fusarium has been isolated from the affected crown, but at present it is not known if this fungus is parasitic or responsible for the death of the plants. This trouble is serious and is causing many growers of strawberries considerable alarm."

Investigations on black root date from this time and are not very extensive. In 1923 Berkeley and Jackson (3) reported the isolation of vari-

ous soil bacteria that they thought to be the causal organisms. Later, in February 1924, Berkeley (4) further reported black root present the second season and doing much damage. The soil bacteria isolated previously failed to cause infection when tested by inoculation into plants. A species of Fusarium was suspected and mycelium was found in sections of diseased roots. In 1924 Sherbakoff (15) reported black root as a disease of wide occurrence in the Southern States and also farther north. He stated that it is of considerable economic importance in certain sections. He isolated a fungus from the diseased roots, but was unable to identify it because it remained sterile in culture. Coons (6) also, in 1924, described black root of strawberries in Michigan and stated that the conditions of infection indicated Rhizoctonia as the causal organism. In 1926 Darrow (7) summed up the situation as follows: "There are several troubles known as black root which are caused by fungi or bacteria. No thorough studies of these troubles have ever been made, but their effect is to kill the roots and so shut off the intake of water and dissolved minerals." Again: "Little is known of the cause or control of crown or root diseases of strawberries. They are often very serious and may cause serious injury which is not recognized as due to disease. They may even be the most important of all strawberry diseases."

Late in 1926 Wardlaw (22) reported on the Lanarkshire disease of strawberries in Scotland. His description is somewhat similar to the black-root disease in this country. He isolated a Pythium and an unnamed member of the *Fungi Imperfecti* that, upon inoculation, produced typical symptoms of disease. In 1927 Ball, Mann, and Staniland (2), reporting the strawberry investigations at Long Ashton, England, describe "sudden wilt" of strawberry. Fungi were isolated, but no proof of pathogenicity was obtained. Although the condition of the root system is not mentioned, the general symptoms resemble those observed in black root. In 1928 Thomas (21), in discussing the killing of strawberry roots in New York State, concluded that the root killing may result from a number of causes and that fungi play a minor rôle. Alcock (1), in 1929, stated that the name "Lanarkshire disease" has been used to include several strawberry-root troubles. She described a root rot that she called the "red core root" of strawberry. This disease is, apparently, not the same as black root. In 1930 Plakidas (13) reported on the root rot of strawberries in Louisiana. Several unidentified species of Pythium were isolated, one of which proved to be very pathogenic when used to inoculate healthy plants.

OCCURRENCE OF THE DISEASE

The black root of strawberries is widely distributed in the United States and Canada. The disease occurs in Mississippi, Louisiana, Tennes-

see, Alabama, New York, Ontario, California, and Washington. On the trip made by the 5th Annual Meeting of The American Phytopathological Society (9) in 1923, through western New York and Ontario, Canada, it was observed at various places.

Collections in Michigan have shown the disease widely distributed in the strawberry-growing districts of the State. It is evident that this disease is extremely wide-spread and coincident with the culture of strawberries. Since strawberry plantations are established with nursery plants, and new varieties are constantly being introduced by nurseries, one might suspect that we are dealing with a disease of strawberries distributed perhaps on nursery stock and thus becoming introduced and established in the fields.

Opposing this view is the fact that plants of native species have been found either killed out entirely or showing marked evidences of the disease by the presence of lesions and rotted roots. These wild plants have been found in many places in the State, and many cases have been observed in locations where strawberries have never been grown as a crop. It will be remembered here that wild-strawberry patches arise from seedling plants, the seeds having been carried by birds. This situation, together with the wide-spread distribution of the disease, strongly indicates that the disease occurs more or less irrespective of soil types, and the cause is to be sought among the local soil organisms.

THE NATURE OF BLACK ROOT

As has been said, examinations of the roots of plants affected with black root showed rotted roots and browned and blackened areas of varying size. (Fig. 3, A, B, and C). These lesions indicate a parasitic organism as the cause.

It has been pointed out that the situation was confused by drought and winter injury. In order to determine whether we were concerned with a disease caused by some parasitic organisms and not by soil or climatic factors, a series of preliminary experiments were performed.

Dead and dying plants typical of the black-root condition were dug up and their root systems chopped into small pieces. This mass of débris was placed in pots so that strawberry plants could be grown with new roots passing through this material. A series of pots were filled two-thirds full of clean sand, a 1-inch layer of débris added, and the pot then filled with sand. As a control, pots of clean sand with no black-root inoculum were employed. Also, pots containing a layer of débris, previously sterilized by autoclaving at 15 lbs. pressure for 30 minutes, were used as additional controls. Young runner plants with root tips just showing were set into these pots. Frequent waterings were made to keep the sand and débris

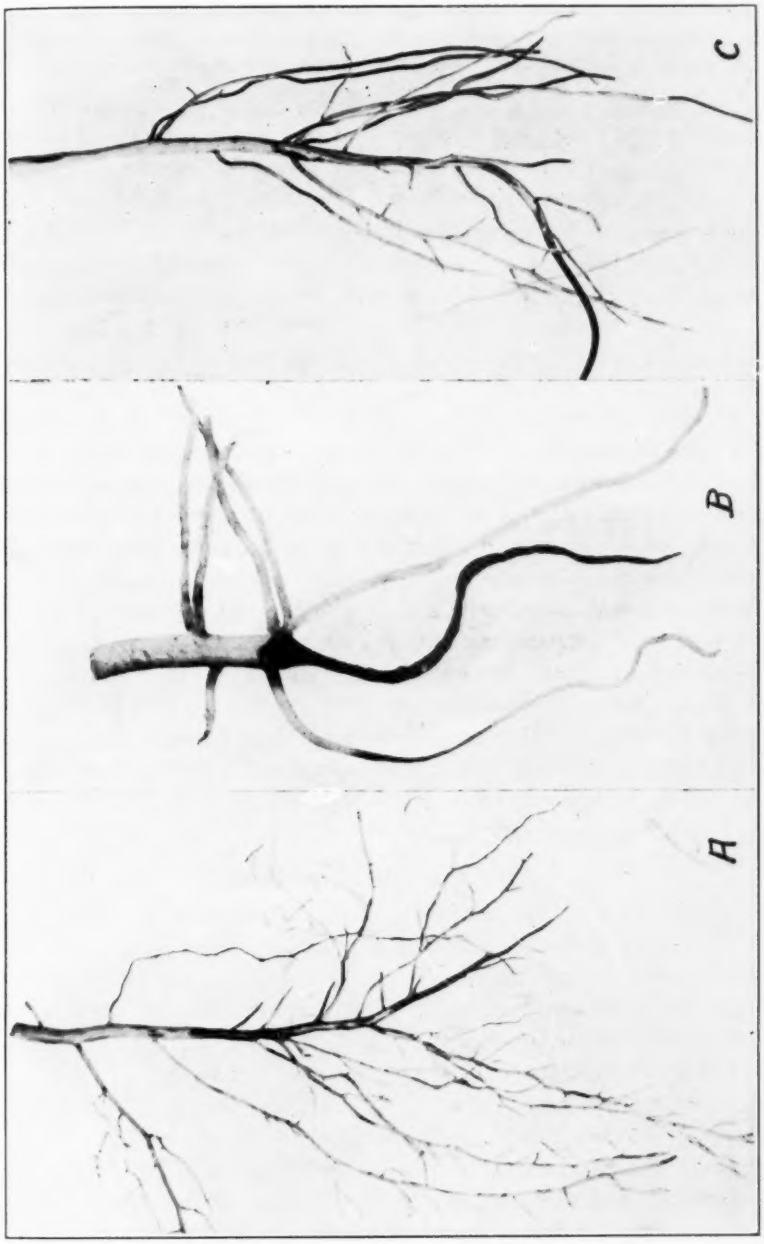

FIG. 3. A. Typical lesions on main root with many lateral roots infected. B. The rotting of a main root from the tip (×3). C. Infected root showing characteristic absence of fibrous roots and many lesions with healthy root tissue adjoining.

inoculum moist. Examinations and readings of the condition of the roots were made in about 14 days. The result of the test as shown in table 1 was

TABLE 1.—*Results of inoculation of clean runner plants with strawberry roots naturally infected with black-root disease*

	Total no. plants	No. plants infected	Infection per cent	Total no. lesions	Average no. lesions per plant
Inoculated: clean sand plus black-root material	32	29	90	104	3.2
Control: clean sand	7	0	0	0	0
Control: clean sand plus autoclaved black-root material	13	1	7.6	3	0.2

conclusive. In this experiment typical black root appeared on 29 out of 32 inoculated plants, while, in the sand controls, all the plants remained healthy, as evidenced by their white roots. The 13 plants inoculated with sterilized débris showed 12 plants healthy, while 1 plant showed 3 lesions. A plausible explanation of this is that it may have been natural infection due to presence in a diseased field and in an open pot. The great number of lesions appearing on the roots of inoculated plants as compared with the clean plants in the sand controls, as well as the plants inoculated with sterilized débris, indicates quite clearly that the disease is infectious and transmissible from one plant to another and parasitic in nature rather than attributable to some soil factor or to winter injury. Examination with the microscope of sections made from diseased roots gave abundant evidence of septate hyphae in the root tissues.

ISOLATION OF THE CAUSAL ORGANISMS

Many plantings were made of bits of blackened roots in Petri dishes containing nutrient medium. Material for use in making such plantings was procured partly from strawberry plants sent in to the Botanical Section of the Experiment Station for disease diagnosis and partly from plants collected by the writers and others of the botanical staff in various parts of Michigan where strawberries are grown extensively.

Prune agar was commonly used as the medium for isolation purposes since its slightly acid reaction discourages bacterial growth. Potato-dextrose agar, corn-meal agar, and strawberry-root agar also were used. The technique of making these plantings consisted in washing the roots carefully to remove the dirt. They were then dried of excess moisture on sterile paper toweling and split lengthwise, the splitting being initiated merely by sterile needles or forceps. Plantings were made from the in-

terior tissues now exposed and that had not been touched by anything in order that no contamination might be introduced. Washing the roots with disinfectants was unsatisfactory, since no growth resulted from plantings made from materials so prepared.

The range of material employed and the number of plantings made are believed by the writers to be extensive enough to afford a fair sampling of black-root material as it occurs in Michigan and to indicate the organisms chiefly involved. The isolations from diseased tissue were extended over a period of 6 years and involved the making of more than 3,500 isolations from diseased plants collected in various parts of the State. The fungi appearing most often in these plantings were: a species of Coniothyrium and a fungus tentatively identified as a species of Gloeosporium and later as a species of Patellina (20) but that we now believe to be identical with the *Patellina fragariae* of Stevens and Peterson (17) and *Hainesia lythri* (Desm.) v. Höhn. shown by Shear and Dodge (14) to be one of the imperfect stages of *Pezizella lythri* (Desm.) Shear and Dodge. Other organisms appearing occasionally were: a brown Fusarium, a red Fusarium, *Pythium* sp., *Acrostalagmus* sp., as well as various species of Mucor and Penicillium.

The Hainesia was isolated also several times from sporodochia on the lower leaves and from the inner tissue of black, shriveled peduncles and petioles of strawberry plants showing heavy natural black-root infection. These plants were badly wilted, and finally died with green berries hanging on the black and shriveled peduncles. The lower leaves were dark brown with abundant cream-color sporodochia, and the root system presented the typical appearance. The roots were all black and rotted their entire length, so that many of them broke off when the plants were removed from the soil.

PATHOGENICITY TESTS OF SUSPECTED ORGANISMS

Following the isolation of the *Coniothyrium* sp. and *Hainesia lythri* in plantings from diseased tissue, these organisms were secured in pure cultures and tested by repeated transfers. Several series of inoculation experiments were set up to test the pathogenicity of these isolations.

Inoculation of Strawberry Seedlings.—The *Coniothyrium* sp. and *Hainesia lythri* were used to inoculate strawberry seedlings grown in deep culture dishes on sterile sand, kept moist with sterile Knop's nutrient solution. Both organisms were able to infect strawberry seedlings. The result of several series of seedling inoculations are arranged in summarized form in table 2. Although several control plants died during the course of the experiment, these plants showed no fungi, externally nor internally. This is thought to be the natural death of less vigorous plants due to lack of nutrition.

TABLE 2.—*Summary of results of inoculations of strawberry seedlings with Coniothyrium sp. and Hainesia lythri*

Inoculated with *Hainesia lythri*			Inoculated with *Coniothyrium sp.*			Control		
No. of plants	Time	Plants infected	No. of plants	Time	Plants infected	No. of plants	Time	Plants dead
			12	13 days	12	46	13 days	0
21	11 days	20	24	13 days	21	42	11 days	2[a]
195	21 days	184	383	21 days	363	111	21 days	16[a]

[a] These plants showed no fungi, externally nor internally. This is thought to be the natural death of less vigorous plants due to lack of nutrition.

Some time after the infection of those plants inoculated with the *Coniothyrium* sp., pycnidia appeared on the roots, stems, and seed leaves. The pycnidia were submerged in the tissue of the host.

FIELD TESTS OF PATHOGENICITY OF CONIOTHYRIUM SP. AND HAINESIA LYTHRI FOR STRAWBERRY ROOTS

The next series of inoculations were undertaken in the field, where runner plants were set in pots filled with clean sand, the purpose being to secure the natural conditions of plant growth and yet avoid as much as possible the contamination from the soil in which the mother plants were growing. In this way it was sought to demonstrate that the two organisms shown to be capable of producing black root under laboratory and greenhouse conditions could produce the field form of black root. For the most part, Premier and Senator Dunlap were the varieties used in these tests. The runner plants remained attached to the mother plants throughout the entire period of the experiments. The former were selected before any roots had started, were treated with a 4 per cent chlorazene solution for 5 minutes, and set so that all root production was made in pots of clean sand. The pots were watered often enough to keep the sand constantly moist.

The inocula consisted of pure-culture material of the *Coniothyrium* sp. and *Hainesia lythri* grown on various media, such as prune juice, potato-dextrose agar, and corn-meal mush. In the first of this series of inoculations, spore suspensions were used. Later, mycelial masses were used in small quantities, and it was found that the inoculations giving the highest

percentage of infection in the shortest time were those in which large pieces of mycelial mats were used. In some experiments the inoculum was placed in the sand, and the roots were allowed to strike and grow down through the inoculum. In other experiments the roots were allowed to strike in the clean sand, and the inoculum was added when the roots were at least 1 inch long. The plants were then removed from the sand and reset. The control plants were handled in a similar manner except for inoculation.

The disease developed in 10 to 21 days. At intervals of about 2 and 4 weeks the plants were removed from the pots and examined. They were then either carefully reset so that later readings could be made or were brought to the laboratory and photographed, and reisolations were made from the lesions.

During the course of this investigation, many such tests were made, all of them giving conclusive evidence of the pathogenicity of the two organisms. The data of these tests, being too extensive to be given in full, are summarized in table 3.

The type of experiment possible under field conditions, if one desires to work with plants as nearly normal as possible, is one in which a medium free from infectious material is used for rooting the runners. The possibility of chance introduction of organisms on the runner plant, from dust and rain splashings, is considerable, and proper conclusions from experiments with soil organisms, such as those considered here, must be based on the relative amounts of infection in inoculated plants and in the similarly handled controls.

It will be noted from a study of table 3 that, in general, the control plants show a small amount of infection that is to be regarded as casual infection, practically unavoidable in field work of this nature. Comparison of the infection in the control plants with the far greater infection found in the inoculated plants, as shown in columns 6, 11, and 16, table 3, the writers believe to be conclusive evidence of the pathogenic nature of the two organisms under consideration.

The data given in columns 3, 4, 5, 8, 9, 10, 13, 14, and 15 of table 3 are from actual records made in the field. The figures in columns 6 and 11 are obtained by dividing the total number of lesions by the total number of plants inoculated. The figures in column 16 are obtained in like manner for the control series of the experiment. The 7th and 12th columns show the relative percentage of infection in the inoculated plants of a series that is properly to be attributed to the inoculation. These percentages were obtained in each series by finding the difference between the average number of lesions per plant in the control and the average number of lesions per plant in the particular series of inoculated plants considered. This

TABLE 3.—*Summary of results of inoculations of clean runner plants, under field conditions, with Coniothyrium sp. and Hainesia lythri*

		Coniothyrium sp.					*Hainesia lythri*					Corresponding controls			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Date of inoculation	No. days duration	Total no. plants	No. plants infected	Total no. lesions	Av. no. lesions per inoculated plant	Infection due to inoculation per cent	Total no. plants	No. plants infected	Total no. lesions	Av. no. lesions per inoculated plant	Infection due to inoculation per cent	Total no. plants	No. plants infected	Total no. lesions	Av. no. lesions per control plant
1926															
July 10A	10	24	11	26	1.08	70	14	5	22	1.57	80	25	6	8	.32
"	27	24	20	127	5.29	87	14	12	104	7.42	91	[a]6	3	4	.66
July 10B	27	24	13	65	2.70	75	40	27	104	2.60	75	[a]6	3	4	.66
July 21	14	31	5	13	.42	88	54	5	11	.20	75	[b]39	2	2	.05
"	37	31	31	560	18.06	85	54	43	350	6.48	59	47	35	126	2.68
July 22	11	33	32	376	11.39	99	25	13	72	2.88	98	[b]39	2	2	.05
August 2	11	10	10	143	14.30	100	13	12	94	7.22	100	[c]9	0	0	0
"	29						13	13	113	8.69	72	[d]9	7	22	2.44
August 4A	8	18	14	145	8.05	100	19	16	117	6.15	100	[c]9	0	0	0
August 4B	27						6	6	75	12.50	80	[d]9	7	22	2.44
August 17	14						16	16	218	13.62	97	29	5	11	.37
August 24	14						18	16	142	7.88	71	27	12	62	2.29
October 2A	26	6	6	100	16.66	92	9	9	126	14.0	90	[e]23	10	32	1.39
October 2B	26	10	10	117	11.70	88	10	10	71	7.10	80	[e]23	10	32	1.39
Aug. 1929	22						85	39	123	1.44	88	41	3	7	.17
Aug. 1930	30	95	83	385	4.05	84	100	93	540	5.40	88	104	26	69	.66
		Total 251	Total 219	Total 2018	Average 8.04	Average 88.2	Total 409	Total 313	Total 2155	Average 5.27	Average 81.9	Total 359	Total 109	Total 343	Average 0.95

[a], [b], [c], [d], [e] Repetition, not separate series.
The two records in each brace represent readings, at different dates, on the same experiment.

difference, divided by the average lesions per plant for this particular series of inoculations, gives the percentage of infection that may reasonably be attributed to the inoculation. Expression of the results in this manner avoids the distortion that would arise if the percentage of infection were based on the number of plants showing lesions on the roots. A control plant showing one lesion would be given equal weight with an inoculated plant that might have 10 to 20 or more lesions.

Sterile sand was used in some of the inoculation experiments, but the amount of infection appearing in the control plants was not decreased by this procedure, indicating that the infection was due to infectious material, blown, or splashed by water, into the sand from outside the pot. Isolations made from lesions on control plants showed a large percentage of the two organisms under consideration.

The lesions and rotted roots found and recorded on the inoculated plants were identical in appearance with the lesions and rotted roots found on plants growing in cultivated strawberry fields and on wild plants. These lesions at first are brown, shriveled areas usually encircling the root and having white healthy root tissue adjoining. These lesions were observed to lengthen until an entire root was involved. The particular organism used in the inoculation was recovered many times from such lesions. Typical infection produced by inoculation is illustrated in figure 4, A and C, while B shows a typical control plant.

INOCULATION OF FRUIT

Ripe berries were carefully washed and placed in sterile moist chambers. They were then inoculated with spores or bits of mycelium, the epidermis of the berry being punctured. The Hainesia caused a rapid leathery rot of the fruit with the production of abundant fruiting bodies (Fig. 5, A), the spore heaps being of a cream or strawberry color. This closely resembles the rot described by Stevens and Peterson (17) for their *Patellina fragariae* and the rot caused by *Hainesia lythri* described by Shear and Dodge (14).

The *Coniothyrium* sp. caused a soft rot of the fruit, but no fruiting bodies were produced.

ORGANISMS INVOLVED

The organisms, shown by the writers to have been concerned in the black-root disease of strawberries, are the imperfect stages of two Ascomycetes. The Coniothyrium species is morphologically very similar to *Coniothyrium fuckelii* Sacc., shown by Stewart (18) to be the conidial stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc. The spore size is within the limits given by various authorities for *C. fuckelii* on other

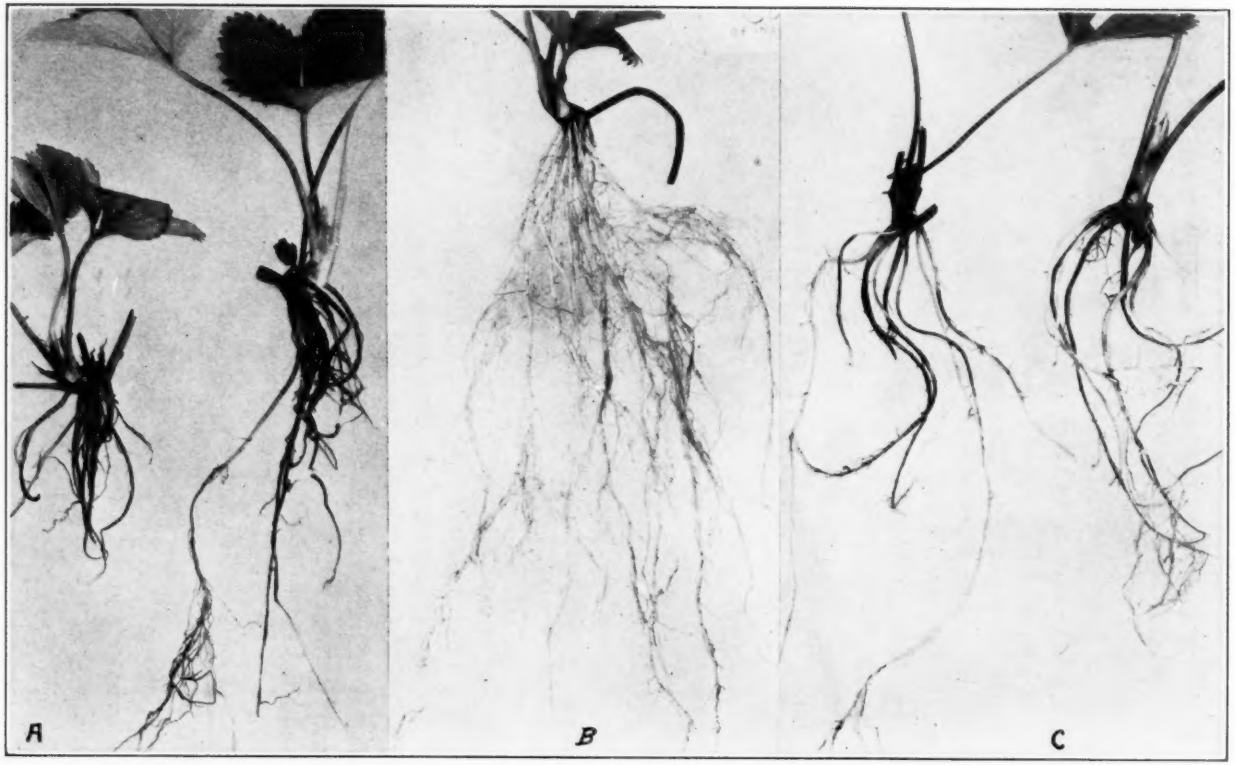

FIG. 4. A. Plants infected by inoculation with *Coniothyrium fuckelii*. The right side of the larger plant is so rotted that many of the roots are broken off. B. Typical control plant. C. Plants infected by inoculation with *Hainesia lythri*.

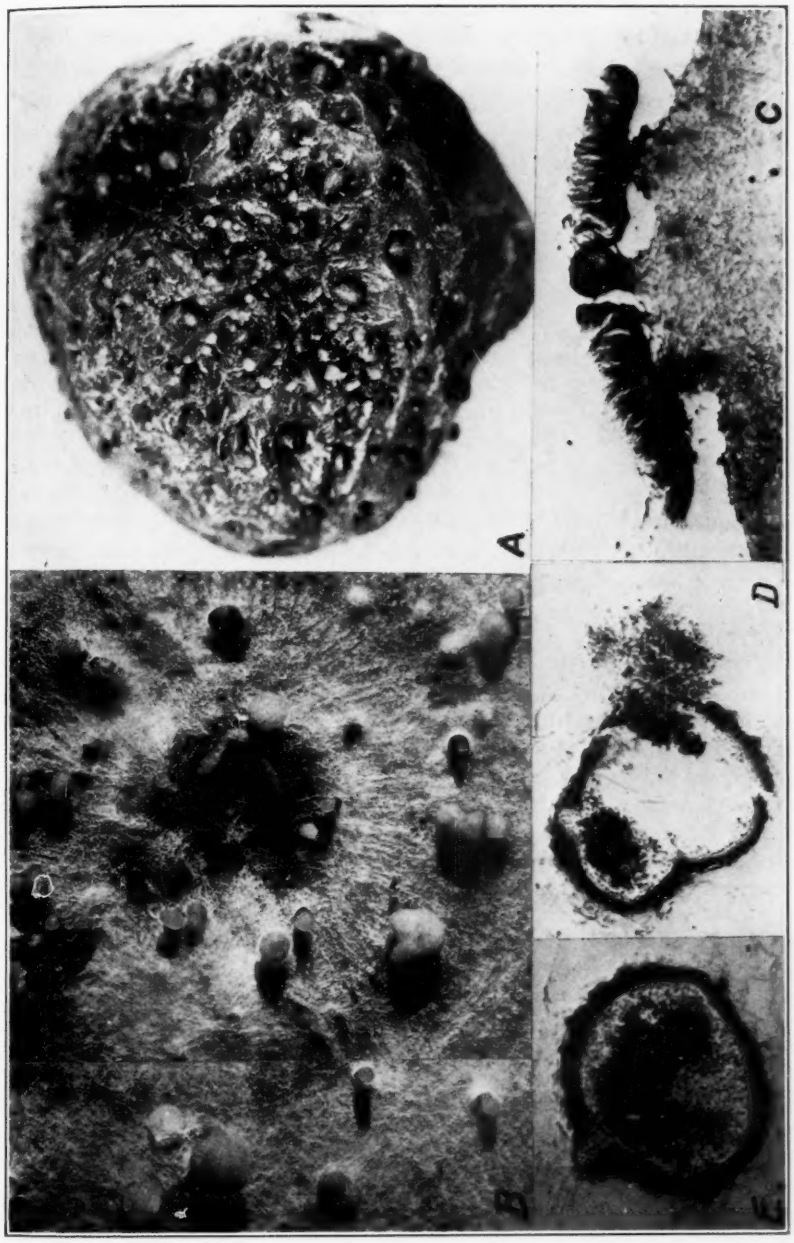

FIG. 5. A. Sporodochia of *Hainesia lythri* on strawberry (×3). B. Several types of sporodochia of *Hainesia lythri* on potato-dextrose agar (×10). C. Vertical section of sporodochia of *Hainesia lythri* showing stipe (×80). D. Section of pycnidia of *Coniothyrium fuckelii* showing spores (×160). E. Section of pycnidium of *Coniothyrium fuckelii* showing ostiole (×200).

Rosaceous hosts. The strawberry black-root Coniothyrium apparently is not the same as *C. fragariae* Oud., which is described as having considerably larger spores (11.6 × 9.3 μ). Cultural and histological studies indicate that the fungus, first considered a *Gleosporium* sp., is identical with the *Patellina fragariae* of Stevens and Peterson (17) and *Hainesia lythri* (Desm.), v. Höhn., shown by Shear and Dodge (14) to be the same fungus and one of the conidial stages of *Pezizella lythri* (Desm.) Shear and Dodge. The following descriptions record the salient characteristics of these organisms.

Coniothyrium stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc.

The pycnidia are ovoid to pyriform with a definite round ostiole, without prominent lips; black when filled with spores, but appearing dark brown after the spores are discharged. Walls thin, almost membranaceous. Pycnidia 160 μ to 200 μ in height and 140 μ to 170 μ in diameter. (Fig. 5, D and E).

Spores exuding in an inky black mucilaginous mass that accumulates at the apex of the pycnidium. Seen singly the spores are fuliginous, unicellular, short, ellipsoid to ovoid, round at both ends and usually 2-guttulate. Size 3.8–4.8 μ × 2.7–3.7 μ, average 4.2 × 3.1 μ.

Hainesia stage of *Pezizella lythri* (Desm.) Shear & Dodge.

The fruiting bodies of this fungus vary in shape from the sporodochium typical of Tubercularia to a cup-shape, stiped body resembling an apothecium. In some cases the cup is nearly closed so that the exuding spore mass forms in a spore horn suggesting the true pycnidial type of fruiting body. Sporodochia on the tissues of the strawberry plant, 70–130 μ in diameter. In culture, often much larger, reaching a size of 700 μ (Fig. 5, B). The wall of the cup is 5 or 6 cells thick, dark brown especially in the basal portion. Sometimes the cups are nearly sessile, although many of them have very pronounced stipes (Fig. 5, C). Spores are produced singly on simple or sparsely branched conidiophores. Early conidiophores are short, but, as the mass of slime-embedded spores increases, the later conidiophores elongate to penetrate this mass. Conidiophores hyaline, slender 20–60 μ long (Fig. 6, A). Spores hyaline, unicellular, slightly curved, oblong with pointed ends, 6.6–9.9 μ × 1.8–2.2 μ (Fig. 6, B). In mass the spores are cream-color when young to brown with age. On ripe strawberry fruits the spore masses are often strawberry-color.

The pycnidial and perfect stages described by Shear and Dodge (14) have never been seen by the writers, either in culture or on strawberry plants, but the organism in question resembles very closely the description and figures they give for the conidial stage of *Pezizella lythri*.

FIG. 6. A. Camera-lucida drawings of conidiophores of *Hainesia lythri* (×700).
B. Drawings of mature spores of *Hainesia lythri* (×700).

ROOT-TREATMENT EXPERIMENT

Along with the work of isolating the causal organisms and determining their pathogenicity, attempts were made to find a root treatment that would be effective in checking or controlling this disease. Mercuric chloride, formaldehyde, and two organic-mercury compounds, Semesan and Uspulun, were used. These compounds were dissolved in water in various concentrations, and the plants were plunged up to the crown in the solutions for periods of 30 minutes before setting in the experimental field. Each treatment appeared six different times in the field, nine rows apart. Every third row was set with nontreated plants as a control. Results do not warrant further details of the experiment, since lesions and rotted roots were found to be as abundant on the treated plants as on the nontreated. The control plants, for the most part, made more vigorous growth than the treated plants.

DISCUSSION

Evidence is presented of the parasitic nature of the black-root disease of strawberry, and the proof is given for assigning the disease to two organisms, *Coniothyrium fuckelii* and *Hainesia lythri*. The rôle that climatic factors play in augmenting the effects inaugurated by these organisms has been mentioned. Inoculation experiments have shown that the two fungi described in this paper are capable of infecting healthy, uninjured strawberry roots, as well as roots that had been injured by removal from, and resetting in, the sand in which they were grown. Thus the inoculation experiments are comparable to the conditions of infection both of uninjured plants, well established in the soil, and runner plants that have had the roots injured by transferring to new plantations.

As has been shown, black root is to be found generally distributed in strawberry plantations in Michigan and elsewhere. The two organisms whose pathogenicity has been demonstrated have been isolated from strawberry roots gathered from many more or less distant places. The Hainesia stage of *Pezizella lythri* has also been isolated from rotted petioles and peduncles and from sporodochia on the lower leaves of plants, indicating the possibilities of rapid dissemination of this fungus in strawberry plantations during rainy weather. The similarity of the lesions found on cultivated and wild strawberries makes it seem possible that the wild plants also are affected with a black-root disease of the same nature as that of the cultivated plants. This suggests that these fungi may be soil organisms of wide distribution. The fact that in this study two organisms have been found, each capable of producing typical black-root conditions, together with the fact that the literature of the disease shows other organisms assignable as pathogens, makes it seem probable that the black-root condition in strawberries may be the result of attack by a number of pathogens working singly or together. Both of the fungi described in this article are capable of producing a rot of the strawberry fruit.

The two organisms isolated in this work are known to be widely distributed and to have a wide host range. Shear and Dodge (14) list Rubus, Oenothera, Acer, Epilobium, Cornus, Smilax, Rhus, and Fragaria as hosts of the Hainesia. *Coniothyrium fuckelii* has been shown to be actively parasitic on other Rosaceous species. O'Gara (12) showed that this fungus causes a canker of rose and apple and a fruit rot of apple. Stewart and Eustace (19) proved *C. fuckelii* to be the cause of cane blight of raspberries.

Although some work has been done on the control phase, the treatment of roots has not been successful in checking the disease. Since the organisms causing this disease are wide-spread, root treatment will be useless. Whether these organisms and others that may cause the disease were originally spread by diseased plants that developed in nurseries cannot be said. This is probably not the case, but the growers of plants for sale should furnish as strong, vigorous, and healthy plants as possible. The grower who sets plantations for the fruit crop must select only this kind of plants for setting. Where root rot is severe, it is advisable to follow a one-crop rotation. Strawberries should be planted in soil where strawberries have not been grown for some years or, if possible, where strawberries have never been grown. The leading nurserymen practice this method to obtain as vigorous and as healthy plants as possible. The ultimate control is, as with so many plant diseases, probably to be sought in the selection or breeding of disease-resistant plants.

SUMMARY

1. Black root of strawberry was found from reports in the literature and collections made by the writers to be a wide-spread disease of cultivated and wild strawberries. Its etiology was not well-known.

2. By inoculation with strawberry débris, the infectious nature of the disease was determined and microscopic examination showed constant association of fungus hypae in the lesions.

3. The Coniothyrium stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc. and the Hainesia stage of *Pezizella lythri* (Desm.) Shear and Dodge have been isolated many times from typically diseased roots and their pathogenicity to plants grown under controlled conditions has been proved.

4. In several series of field-inoculation tests, using runner plants rooted in clean or sterile sand, the typical disease has been produced by each organism, thus leaving no doubt of the etiological relations of these two forms.

5. Technical descriptions of these two species of organisms are given.

6. Treatment of strawberry plants with standard disinfectants before setting did not control black root.

7. General control measures, such as would make for strong, vigorous plant growth and rotation of crops, are recommended. Ultimate control of this disease will doubtless depend on selection of resistant strains.

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FURTHER NOTES ON PLANT DISEASES IN PERU[1]

E. V. ABBOTT[2]

Since the publication of an earlier paper (1) on the diseases of economic plants in Peru several diseases hitherto unreported have come to attention, and information regarding the distribution and importance of others has been accumulated, much of which is of general interest. It appears desirable, therefore, to make a further report on the plant diseases of the country.

SUGAR CANE

1. Sugar-cane mosaic in the Carabayllo Valley has been found to be more widespread than at first reported. Few fields are entirely free of the disease and many of them, particularly the old rattoons, show 90 to 100 per cent infection. It was at first thought that eradication offered the most effective means of control, but, in view of the general distribution of the disease on the plantations of this valley and the indifference toward it on the part of most of the planters, eradication appears to be impractical. Control measures should be concentrated rather on the introduction and testing of more resistant varieties in case mosaic should cause the failure of Otaheite or Bourbon, of which there is little indication at present, even where the disease has been present for 8 to 10 years. Field trials of resistant varieties, including several of the P. O. J.'s, have been initiated.

Although surveys have been made in every important cane-growing valley in Peru for the purpose of determining the presence of mosaic, the disease has not been found outside of the Rimac area. A strict quarantine has been placed on the exportation of seed cane from this region to other parts of the country.

2. The mechanical twisted top is occasionally observed affecting Otaheite, but it is of no importance.

COTTON

1. Fusarium wilt is rapidly becoming more serious in the valleys where it occurs, particularly in the Rimac and Carabayllo, and is causing the abandonment of cotton culture on several plantations. Each year the disease appears in fields where it had not been observed before, the spread apparently being due to inoculum carried by irrigation water.

[1] Contribution from the Department of Plant Pathology, Estacion Experimental Agricola, Lima, Peru.

[2] The writer is indebted to Dr. J. C. Arthur for the identification of *Puccinia glumarum*, *P. Pittieriana*, and *Aecidium cantensis;* to Dr. C. L. Shear for *Phyllachora maydis* and *Phoma zeicola;* to Dr. C. D. Sherbakoff for *Fusarium martii-minus* and *F. solani;* and to Dr. Charles Chupp for *Cercospora zebrina.*

Certain varieties of American Upland cotton, developed in the United States for their resistance to wilt, have failed to show promise when planted in wilt-infested soil in Peru. In experiments conducted at Lima during the seasons of 1929 and 1930, Rowden 40, Rowden 2119, Super Seven, Dixie Triumph, and Express all proved to be more susceptible to wilt than the native Tanguis and had the additional disadvantage that, when planted in wilt-free soil, their production was much less than that of the native variety.

These varieties were planted on October 10, 1929, in soil known to be heavily infested with the wilt fungus. Symptoms of wilt began to appear late in December and the first counts were made on January 1, 1930, just prior to the blooming period. The average percentages of wilted plants at different dates during the season are shown in the following table:

TABLE 1.—*Percentage of plants showing infection by Fusarium vasinfectum at different dates during the 1930 season*

Variety	Jan. 1	Feb. 1	Mar. 1	Apr. 1	June 1
Tangius strain 562	3.2	8.8	36.4	41.7	100
" " 762	3.5	13.5	31.3	67.8	100
" " 2072	2.1	8.8	25.1	32.4	100
Dixie Triumph	3.2	17.7	22.4	100.0	Dead
Rowden 40	2.0	15.0	30.0	100.0	"
" 2119	2.3	15.0	28.7	100.0	"
Express	1.3	19.5	27.0	100.0	"
Super Seven	3.0	12.6	22.7	100.0	"

It will be noted that, although during the early part of the season the Tanguis strains showed about the same degree of infection as the American varieties, Tanguis stood up much better as the plants approached maturity. By June 1 all of the American varieties had been killed by wilt and produced no crop of any consequence, while the Tanguis strains continued to mature a large percentage of the bolls that had set, in spite of the fact that all of the plants showed indications of wilt infection. There was, however, considerable shedding in the Tanguis and the yield was materially reduced.

It is customary in most sections of Peru to grow from one to three rattoon crops of cotton from a single planting, and in the rattoons the greater wilt resistance of Tanguis is more marked than in the plant crop. Although the number of plants of this variety emerging the second season may be reduced from 10 to 50 per cent by wilt, the American varieties fail entirely, having been killed by the disease at the end of the first season. Notwithstanding this greater resistance, however, the yields of the Tanguis rattoon crops are materially reduced, and it is their failure that has forced the abandonment of cotton on some plantations.

Because of the failure of Tanguis on heavily infested soils the planters have been forced to turn to other crops, such as potatoes, beans, corn, and alfalfa, none of which is so profitable as cotton would be were it not for wilt. While this change of crops probably will reduce the amount of wilt in the soil, the chances of effectively controlling it by means of rotations are somewhat lessened by the fact that any crop grown must be irrigated, and under the present system of irrigation it is seldom possible to obtain water that has not passed over wilt-infested fields, thus constantly bringing in fresh inoculum.

2. Powdery mildew (*Erysiphe Malachrae* Seaver). This fungus, which has been described elsewhere (3), is prevalent on cotton in the coastal valleys. Although it may sometimes cause partial defoliation, this occurs late enough in the season so that little or no damage results.

3. Areolate mildew (*Ramularia areola* Atk.) has been observed on *Gossypium peruvianum* in experimental plots at the Lima experiment station but not on Tanguis or the American varieties.

4. In 1929 the Alternaria leaf spot killed 5 acres of cotton seedlings near Lima.

CEREALS

1. Stripe rust of wheat (*Puccinia glumarum* (Schmidt) Eriks. & Henn.) occurs not uncommonly in the sierra where wheat is grown commercially, but it was not observed in the fields of the experiment station at Lima where *P. graminis* and *P. triticina* were always present.

Stripe rust is of much less importance in the country as a whole than stem rust. However, it has been observed causing leaf injury in some sections where both stem and leaf rust were absent. In June, 1930, fields of "Australian" wheat (variety not known), growing at an elevation of 13,000 on Cachi-Cachi farm near Jauja, were found in which stripe rust was prevalent and causing some leaf drying, while neither leaf nor stem rust was present.

2. The importance of stem rust in Peru has already been reported (2). While on trips to the sierra wheat-growing sections the writer has been interested in observing the gradual decrease in rust infection on ascending to elevations greater than 8,000 and 9,000 feet above sea-level. At Tarma (8,000 to 9,000 feet) in June, 1930, the percentage of infected plants of the Peruvian variety "Barba Blanca" was 100 per cent and the injury classed as medium. Ascending the mountain on the road from Tarma to the Huancayo Valley (10,000 to 11,000 feet) fields were examined along the way, in which the decrease in the degree of infection was obvious. In the Huancayo Valley the average percentage of infection was less than 30 per cent, the injury being classed as slight, while at Cachi-Cachi farm (13,000 to 14,000 feet) near Jauja not a trace of stem rust was found in fields extend-

ing over several hundred acres. This was found true also in 1929 during a trip to the same section.

A similar condition was observed in southern Peru in February, 1928, although, at that time, it did not appear so significant until found generally true in the wheat-growing sections of the country. Stem rust in the Arequipa Valley (8,000 to 9,000 feet) was extremely severe, the infection in every field examined being 100 per cent); whereas in the Cuzco Valley (10,000 to 11,000 feet) and at Juliaca (12,000 feet) only scattering plants of the same variety of wheat growing at Arequipa were rusted.

It must be remembered, of course, that wheat, growing at different elevations at a given time of the year, is in a different stage of maturity, a fact which might influence the degree of rust infection. February is the harvest month at Arequipa, while, at Cuzco, wheat usually begins to head during the early part of that month and is harvested in May and June. However, the small amount or absence of rust in the fields at harvest time at elevations above about 10,000 feet has been verified by later visits to the Huancayo-Jauja sections, and by reports from farmers in southern Peru, accompanied in some cases by specimens of the straw.

In a previous paper (2) the opinion was expressed that wind-blown spores from the mountainous wheat-growing sections were the source of stem-rust infection on the coast where wheat always suffers so severely from this disease that it rarely reaches maturity. There appear to be three possible sources of infection: (a) presence of an alternate host which bridges the gap from one crop season to the next; (b) survival of urediniospores on old straw; and (c) wind-blown spores from the higher altitudes.

Species of Berberis do not grow along the Peruvian Coast, although they are native to many sections of the sierra. However, infection of these plants by the stem-rust fungus has not been observed by the writer, even where they were growing adjacent to fields of heavily rusted wheat. No evidence has been accumulated to indicate that the species of Berberis native to Peru play a part in the life cycle of stem rust in that country.

The possibility of some other grass harboring the fungus was considered, but a collection of wild-grass rusts in the valleys adjacent to Lima failed to reveal the presence of *Puccinia graminis*. It seems improbable that a secondary host plant is responsible for initiating the epiphytoties of rust that occur when wheat is planted on the coast.

To determine whether the urediniospores survive from one crop to the next on the coast a series of inoculation and germination experiments was undertaken at the harvest season in November, 1928, and continued in 1929. Small sheaves of the varieties Pusa-4, Hope, and Khapli emmer, showing abundant uredinia, were gathered in the field on November 8, when the heads were in the hard-dough stage, and placed in screened cages in a

shaded place near the edge of the field. A few pieces of straw of each were taken to the laboratory to be used as a source of inoculum. Inoculations were made by dusting the urediniospores on seedlings of Pusa-4 showing the third leaf. Pusa-4 was selected because of its ready susceptibility to rust in the field. The small flower pots, in which the seedlings were growing, were then placed in a moist atmosphere under bell jars and incubated at 18 to 20° C. for 48 hours. At the end of that time the bell jars were removed and the pots, including uninoculated checks, were set in an open patio where they received only the morning sun. Readings for infection were made 15 days after inoculation. This inoculation procedure was repeated at the end of 15 and 30 days from the time of collection of the material in the field, with the straw from the cages serving as the source of inoculum.

The viability of the urediniospores from the three varieties at the time of collection was shown by the production of well-developed uredinia on the Pusa-4 seedlings in 15 days. From the material that had been stored 15 days in the cages, however, only weakly developed uredinia or yellowish spots were produced, while at the end of 30 days' storage there was no indication of infection. Similar results were obtained with Khapli emmer collected in the field on November 15 and December 1.

The experiment was repeated during November and December, 1929, and January, 1930. In no case did the urediniospores survive for more than 30 to 35 days, either when the straw was left in the field or placed in cages. Figures obtained with Khapli emmer are typical. The material was collected on January 13, 1930, from plants in the hard-dough stage and placed in cages as before. Field temperatures during the storage period varied from a daily minimum of 25° C. to a maximum of 36° C. The viability of the urediniospores was determined by germinating them in sterile distilled water in moist chambers at 20° C. and checking the results by inoculations of Pusa-4 seedlings at the time of collection and at the end of 10, 20, and 30 days. The germination counts in each case were based on the examination of 1,000 spores 3 to 3½ hours after placing them in the water. The results are shown in table 2.

From an initial germination count of 41.2 per cent the number of viable spores dropped to 20.5 per cent after 2 days and then decreased gradually

TABLE 2.—*Viability of urediniospores of Puccinia graminis tritici after different storage periods*

Days after collection	0	2	4	10	20	30	38
Percentage of germination	41.2	20.5	13.0	9.0	4.4	1.0	0
Infection of wheat seedlings	+			+	Weak		—

until only 1 per cent at the end of 30 days. After 38 days there was no germination.

Checking these results by plant inoculations it was found that numerous, well-developed uredinia were produced by the freshly collected inoculum. At the end of 10 days' storage of the inoculum the resultant uredinia were of normal appearance, although less numerous. After 20 days only scattered, small uredinia appeared, and at the end of 30 days there was no evidence of infection. Similar results were obtained with inoculum from Hope and Pusa-4.

From these results it seems improbable that the urediniospores remain viable from December or January until the following August when the first rust infection usually appears in the field. Attempts to germinate the spores from the old straw which had remained in the field during that time were not successful.

By the process of elimination wind-blown spores from the higher altitudes are left as the most probable source of stem-rust epiphytotics on the coast. While there is no experimental evidence to support this view, it does not seem unreasonable to believe that they cause at least the initial infections when it is considered that wheat is growing throughout much of the year at different elevations in the sierra and that rust spores are carried long distances by winds. In the case of wheat, growing in the Rimac Valley, it would mean a transfer of only about 60 miles. Considerable wheat is grown every year at Canta, 60 miles distant and 8,000 feet higher, the harvest season coming in June, when the crop is approaching the heading stage in the valley below.

3. It is interesting to note that *Puccinia graminis avenae* Eriks & Henn. was collected by the writer only once in Peru, a single heavily infected specimen from an experimental farm at Huaraz (elevation 11,000 feet) being collected in June, 1930. This was on an unnamed American variety.

At the Lima experiment station several American varieties of oats were grown for three seasons, and, although stem rust was severe on adjacent plots of both wheat and barley, it did not appear on the oats. Nor was the disease observed on oats in other sections of the sierra where stem rust was present on both wheat and barley. The fact that oats is a comparatively new crop in Peru and not yet generally cultivated in the cereal-growing regions may account for the limited distribution of the disease.

4. Barley stripe (*Helminthosporium gramineum* (Rab.) Eriks.) and spot blotch (*H. sativum* P., K., & B.) are common on barley in Peru, the former sometimes causing premature death of the plants and resulting in reduced yields. The severe injury attributed to *H. teres* Sacc. in a previous paper (1) was later found to be due to *H. gramineum*. Net blotch occurs in Peru, but it is of less importance than stripe.

Helminthosporium sativum presents a particularly serious problem in Peru because of the severity with which it attacks Khapli emmer, the only cereal of the wheat group that, up to the present time, has shown any possibility of commercial cultivation on the coast, stem rust making impossible the cultivation of any of the 110 varieties of durum and common wheats thus far tested. During the seasons of 1928, 1929, and 1930 some experimental plots were so severely blighted that they failed to mature. The fungus was isolated from lesions on roots, nodes, culms, leaves, glumes, and seeds.

5. *Helminthosporium oryzae* Br. de H. has been identified as the cause of "black point" of rice in several valleys of the coast.

6. *Phyllachora maydis* Maubl. is common on corn on the coast and sometimes is severe enough to cause premature drying of the leaves.

7. *Phoma zeicola* E. & F. is of rare occurrence on corn.

POTATOES

1. Late blight (*Phytophthora infestans* (Mont.) de By.) is of less "universal" occurrence in the country than was believed at the time the previous report (1) was made. It has since been determined by personal observation that essentially all of the damage attributed to this disease in the sierra is actually due to low temperatures. Local growers do not always recognize this fact and give descriptions of frosted plants which closely approximate the symptoms of blight. The term "hielo" is used for both blight and the effects of cold weather.

In a rather extensive survey of the potato districts of the country during 1928, 1929, and 1930 late blight was not found affecting either wild or cultivated species of Solanum at elevations above about 9,000 feet. In 1928, in the Arequipa Valley (8,000 to 9,000 feet), a small amount of blight was found in some fields. This appears to be contrary to what would be expected if blight were indigenous to Peru, a supposition which had generally been accepted as fact until the appearance of Reddick's paper (5). The disease is present and is often severe along the coast, particularly in the vicinity of Lima where introductions of seed potatoes from Europe and the United States have been made frequently in past years. The fact that blight apparently does not occur in the sections where the potato is native and where introductions of seed stock from other countries have been very infrequent, while the disease is common in the lowlands where such introductions have been made and where the potato is not native, lends some support to Reddick's theory that the blight fungus is not indigenous to South America. That the failure of the disease to develop at the higher altitudes is not due to the resistance of the varieties grown there is shown by their susceptibility to it when grown on the coast.

2. The widespread distribution of powdery scab (*Spongospora subterranea* Wallr.) in Peru has been reported previously (1). The writer has found the disease in every potato-growing section of the sierra visited, which includes the Departments of Arequipa, Cuzco, Puno, Ayacucho, Huancavelica, Junín, Huánuco, and Lima, while specimens were received at the laboratory from Apurimac, Ancash, Libertad and Cajamarca. Not a single field has been examined at harvest time in the Departments mentioned where the disease was not present, usually affecting at least 10 per cent of the crop and frequently 50 per cent or more. Fields on the larger farms, as well as on the small, isolated, mountain-side plots of the Indians, who probably have never grown anything but native potatoes, were affected. It seems to be expected by most growers that at least 5 to 10 per cent of the crop will be discarded each year because of the canker stage of the disease.

The fact that powdery scab is present throughout the parts of the country where the potato is native, particularly in the isolated section of the Andes where it is doubtful whether the Indians have ever had more than casual contact with the White Man, indicates that the disease is indigenous to the country. It is extremely unlikely that an introduced disease would have become so universally distributed throughout the Andean regions. Further support of the belief that it is indigenous was obtained on June 14, 1930, when a wild-potato plant with several tubers showing the characteristic skin lesions of the disease was found on Cachi-Cachi farm near Jauja. The plant was growing on a hillside which had never been cultivated so far as known and about three miles from fields of cultivated potatoes.

3. The presence of wart (*Synchytrium endobioticum* (Schilb.) Perc.) in Peru has been reported (1). From the time the disease was first discovered on Cachi-Cachi farm in September, 1928, a search was made in all potato-growing sections of the country visited in order to determine its distribution. In addition, frequent inspections were made of potatoes in the Lima public markets which had come from many different parts of the country.

No authentic case of the occurrence of wart outside of the vicinity of Cachi-Cachi farm has come to the writer's attention, although a grower from Puno (extreme southern Peru), on examining a specimen of the disease in the laboratory of the experiment station at Lima, stated that it occurred there.

At Cachi-Cachi wart appeared in 1928 and 1929 but was not found in the 1930 crop. The owner of the farm was unable to say how long the disease had been known in that section, but he was of the opinion that it had been there many years. The botanist of the agricultural school in Lima, who has been in Peru for 20 years, stated that Cachi-Cachi is the only place from which he has received specimens of wart.

4. Two rusts (*Aecidium cantensis* Arthur and *Puccinia Pittieriana* Henn.) occur on potatoes in Peru. The former is a new disease described by J. C. Arthur (4) from material collected by the writer in the vicinity of Canta, about 60 miles northeast of Lima, at elevations ranging from 8,000 to 9,000 feet. Although it is quite general on potatoes near Canta during the latter part of the growing season, it is of minor economic importance. It has not been observed outside of the Canta district. The disease also occurs on tomatoes.

Puccinia Pittieriana was collected in February, 1929, near Tarma, where it was causing some defoliation. It was not observed in other parts of the country.

5. Powdery mildew (*Oidium* sp.) occurs generally throughout the sierra, the heaviest infections being observed near Tarma and Canta. Sometimes the stems and petioles are covered by a whitish, powdery growth of the fungus, causing brown patches on the epidermis and often resulting in partial defoliation. The perfect stage of the fungus was not observed.

6. Stem rot and black scab (*Corticium vagus* Berk. & Curt.) are serious on potatoes on the coast nearly every season and frequently cause losses in the sierra. The yellow-flesh variety appears to be particularly susceptible. During the 1929 season some fields of this variety in the Rimac Valley were a total loss as the result of stem rot. No control measures are practiced.

The yellow-flesh variety is one of the choicest of Peruvian potatoes, and, because of its small yield, commands a price two to four times that of other varieties throughout the year. While it does not appear to be a heavy yielder, compared with other varieties, even when free of disease, diseases commonly cause considerable reduction in the yields obtained from it. The most important are stem rot, late blight, and mosaic.

7. Tuber rots, caused principally by species of Fusarium (*F. martii-minus* Sherb. and *F. solani* Ap. & Wr.), prevent the storage of potatoes for any length of time on the coast, particularly during the warmer months. For this reason the coast-grown crop always goes directly from the field to the public markets, and even during the short time intervening between harvesting and consumption considerable numbers of tubers are lost through decay. Cold-storage facilities are lacking. The sierra-grown crop usually is stored in sod houses where the temperature can be kept at a desirable point until market conditions are favorable on the coast.

FRUITS, VEGETABLES, AND MISCELLANEOUS

Diseases of fruits not previously reported include Diplodia rot of oranges (*Diplodia natalensis* Ev.), which has been found infrequently on market fruit in Lima; blue mold of citrus fruits (*Penicillium italicum* Wehmer), which causes considerable loss of fruits in storage and shipment;

peach-leaf curl (*Exoascus deformans* (Berk.) Fckl.), which is serious in some of the sierra valleys, particularly Tarma and Ayacucho, where it is gradually killing small orchards; peach mildew (*Oidium leucoconium* Desm.), general on peaches, but of no economic importance; and a wilt of papaya, with which a species of Fusarium was found constantly associated.

Mosaic is frequently a serious disease of tomatoes. Very little of it was noted in 1928, but, during the 1929 season, losses estimated at 50 to 75 per cent of the crop resulted from it in the truck areas adjacent to Lima. In 1930 losses approached 50 per cent. Mosaic of peppers also is common and often assumes importance.

Miscellaneous diseases identified include *Cercospora apii* Fresen. on celery; *C. citrullina* Cke. on watermelon; *C. zebrina* Pass. on alfalfa; stem rot (*Corticium vagum* Berk. & Curt.) of radish, beet, celery, spinach, strawberry, buckwheat, soy bean, and broadbean; and the Oidium stage of powdery mildews on tomato, eggplant, pepper, cabbage, cauliflower, turnip, radish, cucumber, lima bean, soy bean, velvet bean, broadbean, cowpea, cassava, alfalfa, buckwheat, crimson clover, sour clover, sunflower, sweet pea, and zinnia.

SUMMARY

This paper lists 53 diseases of economic plants not hitherto reported from Peru, together with a discussion of several of the most important ones previously reported.

Sugar-cane mosaic has been found generally distributed on the plantations of the Carabayllo Valley, but it has not been observed in other sections of the country.

Cotton wilt is causing the abandonment of cotton culture in some sections. Field tests are reported in which the Peruvian variety Tanguis proved more resistant to wilt than several North American varieties.

Stem-rust infection of wheat in the highlands was found to decrease gradually with the elevation. Germination and infection experiments are reported indicating that the urediniospores do not survive from one crop to the next, on the coast, supporting the theory that rust epiphytotics on the coast are initiated by wind-blown spores from the sierra. Stripe rust of wheat sometimes causes injury at high elevations where stem and leaf rust are absent.

Potato late blight has not been found in the regions where the potato is native. Its absence from these sections and presence only where foreign seed stock has been introduced support Reddick's theory that it is not indigenous to South America.

Potato powdery scab occurs throughout the Andes where the potato is native and was found once on wild potatoes, indicating that it is indigenous

to the country. Wart was observed in only one potato-growing district. Minor diseases of potatoes reported include a new rust.

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DISTRIBUTION OF CERTAIN FUNGI IN COLORADO SOILS

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The fungus flora of our soils doubtless plays an important part in soil fertility, but in the arid regions of the West little study has been given soil organisms. The soils of Colorado differ greatly in moisture and soluble-salt content and offer a wide range of conditions for growth of fungi.

It is the purpose of this paper to continue the previous report of LeClerg and Smith (3) and give a further survey of the fungus flora from a number of localities and environments in an effort to ascertain the relative frequency of fungus species in this region in soils producing different crops.[2]

MATERIALS AND METHODS

Thirteen different soil types were examined, from which 226 samples were taken.

The methods employed in collecting, preparation of samples, and dilution were the same as described in a previous paper (3). Briefly these are as follows: All samples were taken either with a sterile trowel or a soil auger fitted with a steel sleeve. The sampler was sterilized with mercuric chloride (1:1,000). The top layer of soil, about 1 in., was removed with a sterile spatula before the sample was taken. The samples were carried into the laboratory in sterile, air-tight tin containers. Ten grams of each sample were placed in 300 cc. of sterile tap water and shaken thoroughly. Dilutions were made up to 1:30,000. One cc. of this dilution was poured into a sterile Petri dish, and the plates were poured in the usual way. Waksman's medium was used for all isolation work.

The writer is aware that all the fungi present in the soils examined were not obtained, since special methods are necessary to recover several forms, as Rhizoctonia and Pythium. The plate method for quantitative determinations of soil fungi has its limitations, as it does not distinguish between spores and mycelia. It shows, within limitations, the potential fungus content of the soils examined. The percentages given can not, therefore, be considered as being exact representations of the distributions of the different fungi but are indicative of the relative numbers of each in the different soils under the conditions of sampling, dilution, etc.

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[2] The writer wishes to express his appreciation to Dr. L. W. Durrell for furnishing facilities for this study and for reading the manuscript, and to Dr. J. C. Gilman of the Iowa State College for assistance in identification of the fungi.

DISTRIBUTION AND ABUNDANCE OF FUNGI IN DIFFERENT SOILS

During the course of this work 31 species of fungi were isolated. In confirmation of the previous work (3), species of Aspergillus and Penicillium were more prevalent than any other fungi isolated.

The data in table 1 show that in proportion to the number of isolations the greatest number of fungi found in any of the soil conditions examined was under red-clover plants.

TABLE 1.—*Number of fungi in each of 13 soils*

Kind of soil	Number of samples examined	Number of isolations	Number of species isolated	Total number of fungi isolated (Colonies)
Red clover	14	42	9	1350
Sugar beets	26	78	14	1119
Potato	30	90	10	1114
Wheat	15	45	19	629
Plowed	34	102	16	615
Corn	30	90	21	565
Cherry orchard	15	45	10	437
Gladiolus	10	30	6	431
Below surface (6–12 in.)	8	24	10	244
Alkali	19	57	7	218
Wind-blown	10	30	10	196
Below surface				
(3 ft.)	10	30	4	59
(6 ft.)	5	15	2	24

It is apparent from the data in table 1 that the nature of the cover crop or soil conditions results in modification of the population of soil fungi. The soils examined under such crops as red clover, sugar beets, gladioli, potatoes, and wheat seem to be associated with or make conditions favorable for a relatively large population of fungi. Under such conditions as corn and under cherry trees a smaller population of fungi was found.

The second highest fungal content was in soils producing sugar beets. This agrees quite closely with the results of Starkey (6), who, examining soils supporting apple trees, rye, corn, sugar beets, alfalfa, and eggplant, found the number of fungi to be greatest under alfalfa and second under sugar beets. Jasevoli (2), in Italy, found that soils devoted to potatoes had a higher fungus content than those producing fruit or grain. Similar results also were found in the work herein reported, under conditions in Colorado.

The data in tables 2 and 3, as a whole, suggest that certain crops and soil conditions may affect certain groups of fungi. Corn land contained 21 dif-

TABLE 2.—*Percentage of samples containing various fungi and relation of each fungus to total flora*

TABLE 2.—*Percentage of samples containing various fungi and relation of each fungus to total flora*

Name of fungus \ Soil type number[a]	Percentage of samples containing fungus													Percentage of total flora represented by each fungus											
	1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5	6	7	8	9	10	11	12	13
Absidia spinosa	7									70	80	20	29	b									53	71	3	5
Acrothecium robustum													3													b
Alternaria spp.	17			30	7	79	20	5		10		60	b	2			2	b	5	4	b		5		8	b
Aspergillus clavatus	7		34			7	20					70	3	1		6			b	3					7	b
" *flavus*	3						7							b						b						
" *fumigatus*									13			20	3									b			4	b
" *glaucus*		5							13						2							3				
" *minutus*			11				13									3				8						
" *niger*	23	10	4		27			32						4	b	b		2			2					
" *terreus*	3	7	8											b	b	b										
" *wentii*				1					13								b								12	
Cunninghamella verticillata		3	11				7						3		b	b				1						b
Fusarium spp.	80	100	81	100	100	65	100	63	50	50	60	100	68	44	25	24	15	30	2	23	24	7	39	29	54	36
Gliobotrys alboviridis					1													b								
Hormodendrum cladosporioides	17	20	25	10	27	100	20					10	9	1	2	2	b	3	75	1					b	1
Mucor glomerula	7					21	6							1					2	b						
" *lausannensis*	3								13				3	b								b				b
Penicillium chrysogenum	13					64	27						26	20					1	20						
" *citrinum*	10		4	60	13		13							2		b	38	5		b						19
" *digitatum*	13		42		20		27					40	6	2		48		2		14					12	3
" *echinatum*	7						13		13	10			18	b						3		1	5			7
" *expansum*	20	100	35		87		13	58	88				41	4	54	14		42		14	44	73				17
" *humicola*	10					14	13							1					b	3						
" *purpurogenum*							7													b						
" *stoloniferum*			8													b										
" *viridicatum*	10													2												
Phoma spp.	3						7					10	3	b						b					1	b
Rhizopus elegans	17	60			27		13	11						1	15			1		b	b					
" *nigricans*	50	33	4			28	40	5	25			30	47	6	1	b			b	1	1	2			2	5
Scpedonium chrysospermum			11													1										
Trichoderma lignorum	37	33	19	100	13	36	26	16	13			70	26	5	1	2	40	8	b	1	1	b			11	4

[a] Soil type number 1—Soil supporting corn.
" " " 2— " " potatoes.
" " " 3— " " sugar beets.
" " " 4— " " gladioli.
" " " 5— " " cherry trees.
" " " 6— " " red clover.
" " " 7— " " wheat.
" " " 8—Alkali soil.
" " " 9—Below surface (6–12 in.).
" " " 10—Below surface (3 ft.).
" " " 11—Below surface (6 ft.).
" " " 12—Wind-blown soil.
" " " 13—Plowed soil.

[b] Less than 1 per cent.

TABLE 3.—*Distribution, occurrence, and abundance of fungi isolated from 13 different types of Colorado soils*

Name of fungus	Distribution		Occurrence		Abundance	
	Number of soils containing fungus	Percentage of soils containing fungus	Number of samples in which fungus occurred	Percentage of samples in which fungus occurred	Total number of colonies from 13 soils	Percentage of total flora represented by each fungus
Absidia spinosa	5	38	25	11	86	1
Acrothecium robustum	1	8	1	[a]	1	[a]
Alternaria spp.	9	69	32	14	115	2
Aspergillus clavatus	6	46	24	10	46	1
" *flavus*	2	15	2	[a]	2	[a]
" *fumigatus*	3	23	4	2	11	[a]
" *glaucus*	2	15	2	[a]	27	[a]
" *minutus*	2	15	5	2	88	1
" *niger*	5	38	20	9	100	1
" *terreus*	3	23	5	2	5	[a]
" *wentii*	2	15	2	[a]	30	[a]
Cunninghamella verticillata	4	31	6	3	20	[a]
Fusarium spp.	13	100	181	80	1596	23
Gliobotrys alboviridis	1	8	2	[a]	2	[a]
Hormodendrum cladosporioides	9	69	44	19	1105	16
Mucor glomerula	3	23	6	3	28	[a]
" *lausannensis*	3	23	3	1	3	[a]
Penicillium chrysogenum	3	23	17	8	443	6
" *citrinum*	6	46	22	10	317	5
" *digitatum*	6	46	28	12	641	9
" *echinatum*	5	38	12	5	69	1
" *expansum*	8	62	92	40	1422	20
" *humicola*	3	23	7	3	29	[a]
" *purpurogenum*	1	8	1	[a]	1	[a]
" *stoloniferum*	1	8	2	[a]	11	[a]
" *viridicatum*	1	8	3	1	12	[a]
Phoma spp.	4	31	5	2	11	[a]
Rhizopus elegans	5	38	31	14	186	2
" *nigricans*	9	69	58	26	106	1
Sepedonium chrysosperum	1	8	3	1	14	[a]
Trichoderma lignorum	11	85	67	30	353	5

[a] Less than 1 per cent.

ferent forms comprising 4 different Aspergilli and 7 different Penicillia. It was found that gladiolus soils contained the lowest number of different species of fungi, if consideration be confined to soils supporting only economic crops. In the samples from these gladiolus soils 6 different species of fungi were obtained, including 1 species each of Aspergillus and Penicillium. Species of Fusarium were found to be the most prevalent fungi in all samples taken from wind-blown soils and from soils supporting potatoes, gladioli, cherry trees, and wheat. They were present also in a majority of the samples taken from the other soil types.

From the data in table 3 it is evident that species of Fusarium were widely distributed, occurred more frequently than any other fungus, and constituted 23 per cent of the total flora found in all of the samples. *Penicillium humicola* Oudem. ranked second and *Hormodendrum cladosporioides* (Fr.) Sacc. third. The 8 species of Aspergillus and the 9 species of Penicillium outnumbered any of the other fungi isolated.

On the succeeding pages a discussion is given of the distribution and abundance of fungi in the 13 different soil types examined.

Sugar-beet-producing soils.—The samples of sugar-beet soils were collected from different parts of northern Colorado. Some were taken near Loveland where the soil was white with alkali and very heavy and black; others, east of Greeley; and some, north of Fort Collins.

From the 26 samples examined 14 different species of fungi were isolated. *Sepedonium chrysospermum* (Bull.) Fries was found only in sugar-beet soils. It was present in 11 per cent of the samples but was not abundant, constituting less than 1 per cent of the entire flora in these soils. This fungus has previously been found only twice in the soil, once in England (1) and once in New Jersey (8).

Species of Aspergillus and Penicillium were widely distributed in beet soils. *Penicillium digitatum* Sacc. and *P. expansum* (Link) Thom were most abundant. Fusarium was the most widely distributed fungus in these soils, it having been found in 81 per cent of the samples collected. It constituted about 24 per cent of the entire fungus flora.

Gladiolus-garden soils.—The samples examined from gladiolus soils were taken from fields that had produced plants showing marked signs of root rot (*Fusarium* sp.) the previous year. These fields were located about 10 miles west of Denver, Colorado, in a district where gladioli are extensively grown.

Six different species of fungi were isolated from these soils. Species of Fusarium were found in all the samples examined and constituted 30 per cent of the entire fungus flora. Likewise, *Trichoderma lignorum* (Tode) Harz. was distributed in each sample and formed 40 per cent of the total number of fungi isolated. Species of Alternaria were not abundant (2 per

cent of the total flora) but were found in 30 per cent of the samples. Only one member of the genus Penicillium (*P. citrinum* Thom) was isolated, but it was generally distributed (60 per cent of the samples) and formed 38 per cent of the entire flora obtained.

Corn-producing soils.—Samples were collected in Colorado near Fort Collins, Loveland, Timnath, and Greeley during January and February of both 1927 and 1928. In one field the subsoil was frozen, but the surface layer had thawed during a warm period. None of the samples was taken from frozen soils.

These soils contained more different species of fungi than any soils examined. Twenty-one species were isolated. The total number of fungi found in these soils was small, averaging 6 colonies per isolation.

Species of Fusarium were the most abundant and widely distributed fungi in soils supporting corn. These fungi were found in 80 per cent of the samples and constituted 44 per cent of the total flora. There were 7 species of Penicillium and 4 species of Aspergillus represented.

Potato-producing soils.—The samples of potato soils were all collected on March 13, 1928, at Greeley, Colorado, in two different fields. Part of them (20 samples) were taken from a field that previously had produced a crop of potatoes in which a high percentage of the plants had been attacked by Fusarium wilt and 10 samples were obtained from a field that had produced relatively disease-free potato plants.

Ten different species of fungi were isolated from the 30 samples. *Fusarium* spp. and *Penicillium expansum* were found in every sample examined. The former constituted 25 per cent and the latter 54 per cent of the total flora. The other fungi found were *Aspergillus glaucus* Link, *A. niger* van Tiegh., *A. terreus* Thom, *Cunninghamella verticillata* Paine, *Rhizopus elegans* Eidam, *R. nigricans* Ehrenb., *Trichoderma lignorum*, and *Hormodendrum cladosporioides*. This group of fungi constituted less than 25 per cent of the entire flora.

More colonies of *Fusarium* spp., per culture, were obtained from the soil that had produced a "wilt-sick" crop than from the soil that had produced a crop of only a few affected plants.

Cherry-orchard soils.—Fifteen samples were collected near Loveland, Colorado, on February 8, 1928, from two different cherry orchards. Ten species of fungi isolated from these soils consisted principally of *Fusarium* spp. and *Penicillium expansum*. The former were present in all the samples examined and the latter in 87 per cent of the samples. *Fusarium* spp. formed 30 per cent of the total flora, while *P. expansum* constituted 42 per cent of the total number of fungi found. The remaining fungi isolated consisted of *Alternaria* spp., *Aspergillus niger*, *Gliobotrys alboviridis* v. Höhnel, *Penicillium citrinum*, *P. digitatum*, *Rhizopus elegans*, *Trichoderma lignorum*, and *Hormodendrum cladosporioides*.

Wheat-producing soils.—Fifteen soil samples were collected from widely distributed places in Colorado in fields of winter wheat. Collections were made at Fort Collins, Rocky Ford, Akron, and Greeley.

Nineteen species of fungi were isolated from the 15 samples of wheat soils. Of this number about one-third (7) consisted of species of Penicillium. Species of Fusarium were found in all the samples and constituted 23 per cent of the total flora. Species of Alternaria were found in one-fifth of the samples but formed only 4 per cent of the total number of fungi. Species of Penicillium were widely distributed, and the following were isolated: *P. chrysogenum* Thom, *P. citrinum*, *P. digitatum*, *P. echinatum* Dale, *P. expansum*, *P. humicola* Oudem., and *P. purpurogenum* Stoll. About 33 per cent of the soil flora consisted of *Aspergillus clavatus* Desm., *A. flavus* Link, *A. minutus* Abb., *Cunninghamella verticillata*, *Mucor glomerula* (Bain.) Lendn., *Phoma* sp., *Rhizopus elegans*, *R. nigricans*, *Trichoderma lignorum*, and *Hormodendron cladosporioides*.

Alkali soils.—Some of the alkali soils from which samples were taken had only a vegetative cover of *Sporobolus airoides* Torr., which is indicative of a considerable amount of alkali in the soil. Another field supported a dense cover of *Distichlis stricta* (Torr.) Rydb., which indicates very high alkali content. Nineteen samples were collected from different fields all of which were high in soluble salts.

The 57 isolations from these soils developed only 218 colonies of fungi, or an average of 4 colonies per isolation. This would seem to indicate that the presence of salts in the soil tends to interfere with the growth activities of the soil fungi. Seven different species were isolated from the 19 samples. It is interesting to note that *Fusarium* spp. were very abundant; 63 per cent of the samples contained species of this fungus and constituted 24 per cent of the total flora isolated from alkali soils. *Penicillium expansum* also was abundant, appearing in 58 per cent of the samples and forming 44 per cent of the total flora. Other fungi isolated from alkali soils were: *Alternaria* spp., *Aspergillus niger*, *Rhizopus elegans*, *R. nigricans*, and *Trichoderma lignorum*.

Subsurface soils (6–12 in. deep; 3 ft. deep; 6 ft. deep).—The average number of fungi per isolation decreased with depth; at 6–12 in., 10 colonies were found; at 3 ft., 2 colonies; and at 6 ft., only 1 colony per isolation appeared on the agar medium. Other workers, Russell (5), Paine (4), Taylor (7), and LeClerg and Smith (3), have found this to be the condition in soils below the surface.

The samples taken at 6–12 in. showed that *Penicillium expansum* and *Fusarium* spp. were the most abundant. Other fungi found were: *Aspergillus fumigatus* Fr., *A. glaucus*, *A. wentii* Wehm., *Mucor lausannensis*, Lendn., *Penicillium echinatum* Dale, *Rhizopus nigricans*, and *Trichoderma lignorum*.

Four different species were isolated 3 ft. below the surface. These were *Absidia spinosa*, *Alternaria* spp., *Fusarium* spp., and *Penicillium expansum*. Species of Fusarium and *Absidia spinosa* were found most frequently and abundantly at this depth.

Absidia spinosa and *Fusarium* spp. were isolated at a depth of 6 ft. The former was most abundant.

Wind-blown Soils.—Ten samples of wind-blown soils were collected near Akron and Sterling, Colorado, on May 1, 1928. The soil contained very little organic matter and did not have a vegetative cover.

Species of Fusarium were found in all samples of these soils and constituted over half (54 per cent) of the total fungi isolated. *Trichoderma lignorum*, *Aspergillus clavatus*, and *Alternaria* spp. were very abundant. Besides these fungi 6 other species were obtained, which included *Absidia spinosa*, *Aspergillus fumigatus*, *Penicillium digitatum*, *Phoma* spp., *Rhizopus nigricans*, *Trichoderma lignorum*, and *Hormodendrum cladosporioides*.

Plowed Soils.—Sixteen different species were isolated from various plowed soils. The most prevalent species found were members of the genus Fusarium. *Penicillium expansum* and *Absidia spinosa* were also abundant. The other fungi isolated consisted of *Acrothecium robustum* Gilm. and Abb., *Alternaria* spp., *Aspergillus clavatus*, *A. fumigatus*, *Cunninghamella verticillata*, *Mucor lausannensis*, *Penicillium citrinum*, *P. digitatum*, *P. echinatum*, *Phoma* spp., *Rhizopus nigricans*, *Trichoderma lignorum*, and *Hormodendrum cladosporioides*.

SUMMARY

A study was made of the fungus flora of 13 soils of different physical conditions supporting different crops. Thirty-one species of fungi were isolated. Alkali salts tend to reduce fungus flora, although *Fusarium* spp. were abundant in this type of soil.

Fungi were more abundant in planted than in nonplanted soils, such as wind-blown soils.

The number of fungi was greatest in soils producing red clover.

The only forms that occurred under all soil conditions were *Fusarium* spp. These fungi were more abundant in soils that had produced wilt-sick crops of potatoes than in soils that had produced a crop of only a few affected plants. Likewise, they were very abundant in soils that had produced a crop of gladioli badly affected with Fusarium root rot.

Species of Aspergillus and Penicillium outnumbered any other fungi isolated.

The number of fungi decreased with depth into the soil.

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NODULE PRODUCTION ON ETIOLATED VETCH SEEDLINGS

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Descriptions of the mode of entry of the legume bacteria into the root tissue of leguminous plants have appeared from time to time in the literature. They agree for the most part in details. Apparently, the bacteria produce a small colony near the apical end of a root hair. Here they readily effect entrance through the cell wall and mingle with the protoplasm. Soon they develop an infection thread. This extends towards the main root. By this time, usually within one day, the root hair had enlarged and on certain plants become twisted. Further progress of the infection thread into other cells, together with increased cell division, produces a nodule.

In 1902 Peirce[1] published an article on the root tubercles of bur clover and of some other leguminous plants. In this he described the infection of root hairs on seedlings whose roots were only an inch or so long. The description agreed with that that had been given for the infection of root hairs of other plants. From the experiments performed it was evident that infection occurred before the seedlings were old enough to carry on photosynthesis. This indicated that infection may occur without the stimulus of photosynthesis and that the latter may not be necessary for nodules to develop.

Perhaps the reserve material in the seed sufficed to carry the seedling through the infection period and, if enough assimilable carbon compounds were available, either as reserve materials in the seed or supplied to the seedling, macroscopic nodules would develop in the absence of light. Definite experiments, therefore, were started, the object being to produce nodules on etiolated seedlings.

A medium in which to grow the plantlets contained the following:

$MgSO_4$	0.2 gram
KH_2PO_4	0.2 "
NaCl	1.0 "
$CaSO_4$	0.1 "
Powdered agar agar	15.0 grams
$CaCO_3$	excess
Water	1 litre.

After the above medium was boiled for a few minutes 50-cc. portions were run into test tubes 4 x 16 cm. in size. These were then divided into

[1] Peirce, G. J. The root-tubercles of bur clover (*Medicago denticulata* Willd.) and of some other leguminous plants. Calif. Acad. Sci. Proc., Ser. 3 (Botany) 2: 295–328. 1902.

lots. Each lot received a certain sugar in definite concentrations. The sugars used were dextrose, levulose, and saccharose, and the concentrations were 0.50, 1, and 2 per cent of each, respectively. Twelve tubes were provided without sugar. Each was plugged and all were sterilized.

After sterilization, but before the contents of the tubes had cooled, enough sterile calcium chloride solution was added to make a concentration of this salt in the medium 1/500 molecular. The contents were thoroughly mixed and the tubes placed in an upright position until they were cold.

On October 6 seed of vetch, *Vicia villosa* Roth., were sterilized with a solution of calcium hypochlorite and 5 or 6 of them dropped on the surface of the agar medium in each tube. Two days later some growth from an active culture of *Rhizobium leguminosarum* Frank was suspended in sterile water and a few drops added to each tube. These tubes were then placed in an incubator at 25° C.

No daylight was allowed on the seedlings during the growth period but they were examined periodically under the electric light. Plants grown without access to sugar died much sooner than plants that had access to 0.50 per cent saccharose, while those that grew where 2 per cent saccharose was supplied not only lived the longest but also produced the largest plants. In several instances the plants grew to 30 or 40 cm. in length. All plants were entirely etiolated. Root development also was very good.

It was evident from an occasional examination of the plant roots, which could be seen through the test-tube wall, that infection had occurred and nodules were developing on a few plants. After 36 days, when it was evident that many plants were drying, the test tubes were removed from the incubator and the plants taken from them. The roots were carefully examined. Plants grown without access to sugars showed very few nodules. Out of 40 or more plants only 1 nodule was found. Also where levulose 0.50 per cent concentration, was supplied to the plants only a single nodule was found. Plants with access to dextrose also failed to develop nodules in all the concentrations except that of 2 per cent. Here 1 plant had 2 nodules. Plants grown with saccharose in the medium developed nodules in all the concentrations supplied, more being present in the 0.50 and 1 per cent concentrations than in the 2 per cent concentration. Some plants that were grown in the 0.50 per cent sugar medium had as many as 4 nodules. Figure 1 shows the appearance of the macroscopic nodules of etiolated vetch plants in a tube.

This experiment has been repeated several times with the same results. It has also been tried with peas, alfalfa, and red clover. No nodules were seen on the two latter plants but 2 nodules were found on a pea plant that had grown with access to 1 per cent dextrose. Many hand sections of the nodules from these plants were examined under the microscope. The nodular cells were packed with bacteria.

FIG. 1. Etiolated vetch seedlings showing nodules on roots. Grown for 36 days with 1 per cent saccharose in medium.

The foregoing observations, together with those recorded by Peirce, suggest that infection of leguminous plants by Rhizobium may occur before photosynthesis begins and that, if suitable conditions are maintained by supplying a satisfactory carbohydrate to the seedling, macroscopic nodules may develop in the absence of light.

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PHYTOPATHOLOGICAL NOTES

The Viruses Concerned in a Natural Epiphytotic of Streak in Tomatoes.—While viruses and virus combinations are known which will cause streak in tomatoes experimentally, the identity of the viruses concerned in natural streak has been determined and reported in but few cases. This note is for the purpose of recording the viruses concerned in a natural epiphytotic of streak and mosaic which occurred in the horticultural greenhouse at the Kentucky Agricultural Experiment Station during the fall of 1930. Tomatoes have been grown in this house during the past several years, but no cases of mosaic developed until the past fall. One year a few tomato plants were found affected with the so-called healthy-potato virus, the source seeming to be Cobbler potatoes, growing in the house.

Streak was first noticed about the middle of October, 1930, when the plants were approximately 3 ft. high. It started near one corner of a bench and appeared to spread gradually so that about 4 weeks later, of 51 plants in this bench, 22 were affected with streak and 12 with mosaic. Several plants in the adjoining bench were affected with streak and mosaic. In house 2, separated only by a partition in which is a door, no streak developed, but mosaic was present in many plants.

Inoculations were made to 59 Turkish tobacco plants from each of the 51 plants in the first bench of house 1 and from selected plants in bench 2 and in house 2. The tobacco plants, following infection, could be placed in 4 groups as follows:

Healthy	15
Healthy-potato	3
Tobacco-mosaic	18
Tobacco-mosaic with necrosis	23

The healthy-potato virus was obtained from plants with a very faint but distinct mottle, quite typical of the disease produced when tomatoes are inoculated with this virus from potatoes. The disease in tobacco was characterized by small, concentric rings on rubbed leaves and necrotic ring and line patterns and watered-silk chlorotic patterns in the newer leaves. It was found only in house 1. The tobacco mosaic was obtained from plants having a rather severe mosaic, but no evidence of streak on the stalk or petioles and no leaf necrosis. When dried, the virus retained its infectivity, indicating that it is one of the typical tobacco mosaics. It produced a severe mosaic in tobacco, without leaf necrosis, on rubbed (inoculated) and subsequent leaves. The necrotic mosaic was obtained from some slow-growing plants that appeared to have only tobacco mosaic or mosaic with some leaf necrosis and from rapidly growing plants with

stalk and petiole streak and leaf necrosis. Fruits on these plants developed no mottling nor necrosis. In tobacco, necrotic spots developed on rubbed leaves in a few days, the spots gradually increasing until many of the inoculated leaves died. Gradually the symptoms of typical, severe tobacco mosaic developed in the growing point, accompanied by necrotic dots or small rings. These plants usually were more stunted than those with mosaic only. Mosaic, when transferred to tomatoes, produced mosaic only. The viruses obtained from mosaic and healthy-potato tobacco plants, respectively, produced streak in tomatoes identical with that produced by transfers from the necrotic mosaic-tobacco plants to tomatoes. It was shown in the following ways that streak in the tomatoes and the necrotic mosaic in tobacco were the result of a mixture of tobacco mosaic and the potato virus. 1. A mixture of the two viruses produced symptoms in tobacco and tomatoes identical with those produced by the virus complex from streak tomatoes. 2. Symptoms of the healthy-potato virus could be clearly recognized in tobacco plants affected with the streak virus complex. 3. Transfers of the streak virus complex to virus-free seedling potatoes (previously tested for the healthy-potato virus and found free) produced stem and leaf streak typical of that produced by tobacco mosaic alone, but transfers to tobacco from potato leaves produced subsequent to inoculation resulted in only the concentric rings and other symptoms typical of the healthy-potato virus. 4. Transfers of the streak virus complex to *Datura stramonium* resulted in a mild mottle of the new leaves and transfers from these to tobacco resulted in the healthy-potato disease. Thus the healthy-potato virus was separated from the virus mixture and obtained pure in tobacco. The evidence seems to indicate that these two viruses only were concerned in this natural epiphytotic.[1]

As to the source of the viruses, nothing is known definitely. It is probable that they were introduced into the house independently, as the potato virus was present alone in a number of tomato plants. It is not likely that this would have happened if the viruses had come into the first plant together, as tobacco mosaic is so readily spread that mechanical operations which would spread the potato virus would almost certainly spread the tobacco-mosaic virus. The fact that streak was found in one house and only tobacco mosaic in the adjoining house suggests that mosaic was spread from the first house to the second on the hands of the caretakers, probably while suckering, and that the potato virus was not spread in this way. Tomato plants out of doors were affected with various kinds of mosaic, and,

[1] Following removal of the tomato plants in midwinter, the benches were not reset with tomato plants until about a month later when virus-free seedlings were set. A number of these became infected with tobacco mosaic, evidently from nondecomposed roots of the previous crop, but streak and the healthy-potato disease did not reappear.

as the men who handled these also handled the greenhouse tomatoes, the mosaic virus could have been introduced in this way.

The source of the potato virus and its spread in house 1 are not so readily explained. Potatoes were growing within a hundred yards of the house, and, while we have no evidence of insect spread of this potato virus, yet it is possible that it may have been introduced by insects to house 1 and spread either by man or by insects from plant to plant in this house before the introduction of the tobacco mosaic. Its early introduction seems probable, as the mottle symptoms were found well down on some of the plants, far below those of the streak or mosaic symptoms. Another possible means of introduction was on the hands of the men. The early-crop potatoes, dug at the usual time, rotted in the cellar and had frequently to be picked over during the summer and fall. The men who did this also cared for the tomatoes and may have introduced the potato virus in this way.—W. D. VALLEAU AND E. M. JOHNSON, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

A Rubber Dressing for Tree Wounds.—The experimenter who works with dressings for tree wounds finds himself confronted at the outset of his investigation with the dual nature of the task peculiar to wound dressing. By the application of various paints he hopes to accomplish two distinct purposes: (1) He endeavors to preserve the heartwood against the attack of weather, insect, and fungus. (2) He seeks to protect the region loosely designated as the "cambium" against drying out during at least the initial stages of callus formation, in order to assure prompt closing in of the ring of growing wood and bark that is ultimately to form about the wound. He has thus to deal with both living and dead tissues. Applications to living parts must be restricted to the use of more or less inert materials which do not penetrate the tissues deeply and which are nontoxic to the living cells. In dressing the inner area of the wound it is desirable to attempt the impregnation of the heartwood to some depth with preservatives which will be toxic to biological foes and deterrents to checking. Here, the heartwood being already dead, there is no question of toxicity to the cells.

In general practice the ringing of the cambium region with one material and the painting over of the wound area within with another, while long recognized as theoretically sound, are seldom attempted. It is more troublesome to apply two kinds of dressings than a single dressing. The entire cut generally is painted with a material, primarily designed (1) to preserve the dead tissues even though it injure the growing layer; or (2) to aid the growth of the callus and afford little protection to the heartwood; or (3) to seek a middle ground of giving reasonable protection to the dead tissues with a minimum of accompanying injury to the live.

During the past 4 years the writer tried the effect of a number of substances on the formation of callus with a view to testing their merits for preventing the drying out of the cambium and for protecting it when strong chemicals are applied to the heartwood. But few of the materials tested proved valuable. Shellac is applicable to this purpose, being a non-injurious material. It has the disadvantage of short life when not covered with some more permanent material, sometimes becoming useless through weathering after a few days of exposure. Its protective coating is thin and brittle. Melted beeswax is another beneficial dressing for this purpose. It coats the surface with a reasonably thick, firm layer that neither penetrates deeply nor kills the living cells. Results with it are superior to those obtained with shellac. Like the latter material, it becomes somewhat brittle with age. The fact that it must be kept hot while being applied is a further disadvantage.

From a strictly theoretical standpoint, rubber was thought to offer great promise for such ringing of wounds. One would expect to obtain by its use an elastic coating that would protect the growing layer from toxic preservatives painted onto the heartwood. Some years ago the writer attempted two methods of applying rubber to tree wounds: (1) By the cementing on with shellac of rubber dam similar to that used in dentistry and (2) by painting the surface with liquid-rubber compounds designed for the application of cold patches to inner tubes of automobile tires. Both methods were pronounced failures. The dam became loose, and the benzene used as a solvent in the liquid-rubber compound penetrated and injured the living cells.

Rubber latex,[1] containing vulcanized rubber suspended in a colloidal state, is now available on the market. It is a white liquid having much the color and consistency of thin cream. When the liquid is applied to a surface by means of an ordinary paint brush or a paint sprayer and allowed to dry, a film of cured rubber is formed. This rubber latex was tried last spring (1930) as a dressing for tree wounds. It was applied to cuts immediately after they were made, a single coat being flowed on freely with a paint brush. The material dried quickly and the resulting rubber film seemed relatively impervious, highly elastic, strongly adhesive to the wood, and of great tensile strength. Throughout the past growing season it has resisted the action of weather and the organic substances present on the wounds, and came through in excellent condition. In our experiments on about a dozen species of trees and, when applied under varied weather conditions, this dressing has not injured the cambium region nor retarded growth. In fact, by preventing desiccation of tissues, the production of callus has seemed to be somewhat stimulated as compared with that of

[1] This compound is sold under the name "Vultex" by the Vultex Corporation of America, 708 Main Street, Cambridge, Mass.

untreated wounds. Besides the excellent results given by the material, its cost is reasonable and it is easily applied with a brush or a sprayer without any previous treatment or heating. It may also be used when the temperature is below freezing. The lowest temperature at which it was used by the writer was 19° F. It was not affected by this temperature.

With the availability of a material so well suited to the task of ringing, it is probable that double dressing will become increasingly popular and possibly largely replace single dressings for use on large wounds. The writer does not seek to suggest that the use of this latex be restricted to the ringing of cuts. It already has given most excellent results in the treatment of small wounds. It has proved its merit, when promptly applied, for the dressing of large superficial wounds due to barking, a type of injury frequently inflicted on street trees by automobiles. It holds promise for any who might care to test its application to the various processes of budding and grafting.

This rubber compound warrants inclusion in the trials of all who are investigating the subject of wound dressings for trees. It justifies further experimentation with double, or ringed, dressings despite the increased cost and bother their use occasions. It is a material for which the research of the horticulturist, pathologist, and physiologist may find considerable application.—RUSH P. MARSHALL, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Osborn Botanical Laboratory, Yale University.

Pseudomonas prunicola and Bacterium citriputeale.—The bacterial disease of stone fruit trees, caused by *Ps. prunicola*, recently described from Britain, has many of its cultural characteristics identical with those of citrus-blast or black-pit caused by *Bact. citriputeale*. The writer, through the courtesy of Dr. Wormald,[1] secured a culture of his Prunus organism, *Ps. prunicola*. Its growth on dextrose-potato agar resembles closely that of the citrus-blast organism, and inoculations by it through wounds in nearly ripe lemon fruits that were kept in a moist chamber produced within 10 days typical black pit-like lesions identical in appearance with those that have been produced with the citrus-blast, lilac, and Prunus organisms that have been isolated from California material.[2] It seems that *Ps. prunicola* is apparently very similar to, if not identical with, the lilac organism, *Bact. syringae*, with the citrus-blast organism, *Bact. citriputeale*, and with the Prunus organism, *Bact. cerasi*, as reported from Oregon and California.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[1] Wormald, H. Bacterial diseases of stone-fruit trees in Britain. II. Ann. Appl. Biol. **17**: 725–744. 1930.

[2] Smith, Clayton O., and Howard S. Fawcett. A comparative study of the citrus blast bacterium and some other allied organisms. Jour. Agr. Res. **41**: 233–246. 1930.

BOOK REVIEW

Sorauer, Paul. *Handbuch der Pflanzenkrankheiten, Band II, Fünfte Auflage.* Herausgegeben von Dr. O. Appel, Dr. Paul Graebner, und Dr. L. Reh. 758 pp. Paul Parey, Berlin. 1928.

As pointed out by Dr. Appel in the introduction to this volume, Sorauer's Handbuch has become not only one of the classics in plant pathology, but the successive editions mirror in a unique way the progress of the science. The preponderating emphasis on predisposition and non-parasitic diseases in the earlier editions and the increasing emphasis on parasitic diseases and pathogens in the later ones reflect to some extent the transition from the autogenic to the pathogenic theory of the cause of plant disease, although the influence of environment on the development of diseases is by no means neglected in the last edition. Indicative of the progress in knowledge is the fact that the first 295 pages of volume 2 of this 5th edition are devoted to bacterial diseases, whereas in the 4th edition only 86 were devoted to this subject.

The book is divided into two parts: the first deals with diseases caused by bacteria, and the second with those caused by Myxomycetes, Phycomycetes, and Ascomycetes.

The section on bacterial diseases is thoroughly done. Following an introduction, in which certain generalities and principles are discussed, there is a classification of bacteria according to Migula's System. The diseases are then grouped according to orders of host plants. This arrangement has certain obvious advantages and disadvantages, although, in the case of bacterial diseases, the former outweigh the latter. The disease symptoms are thoroughly, sometimes almost minutely, described, the historical summaries are useful, the organisms are described in detail, and the pathological anatomy, contributing factors, and economic importance and control measures are discussed in some detail for the most important diseases. The section is an excellent compilation of our knowledge of bacterial diseases up to 1926; the important literature, for the most part, has been summarized and evaluated with judgment and skill. The references are given in footnotes, which are convenient in reading, but have the disadvantage of not presenting an easily available bibliography. The section is a very commendable and useful summary, written in a style that should not be difficult for English-speaking scientists with a fairly good knowledge of German. The principal criticism, in my opinion, is the lack of adequate headings. For example, one finds on page 121 the heavily leaded center head, "24. Bakteriosen der Rosaceen." The description of diseases of this group ex-

tends to page 159, but the absence of headings may prove inconvenient for a busy reader.

In Part II the diseases caused by slime molds, Phycomycetes, and Ascomycetes are discussed. The arrangement is according to the taxonomic position of the pathogens. There is a certain lack of uniformity in treatment and viewpoint, as would be expected from the fact that different sections were prepared by different contributors. In general, one is impressed by the comprehensiveness of mycological and pathological information presented, although in certain sections the mycological viewpoint seems to predominate over the pathological. This probably is almost unavoidable in a book of so wide a scope, where an attempt is made to mention, briefly at least, virtually all pathogens. The mycological proglems involved are, of course, also sometimes puzzling and the attempt to elucidate them is commendable, although the strictly pathological phases are only rather briefly discussed in a few instances. In general, the balance between various phases is good. The headings in Part II are helpful, and, for the most part, well arranged.

Mechanically, the book is good. It is printed on good paper; the type is clear; the illustrations are mostly good, although a few more, illustrating certain diseases, would be helpful; and typographical errors are no more numerous than would be expected.

The prodigious amount of work required in compiling a book of this kind would cause a critic to be lenient, even if there were more in the book to criticize adversely. Those responsible for the book deserve rich credit, not only for bringing together in one volume such a tremendous amount of widely scattered information but also for excellent perspective in the treatment of the material.—E. C. STAKMAN, University Farm, St. Paul, Minn.